

Figure 1

TRAIL induced apoptosis in Jurkat Cells

12 HOURS

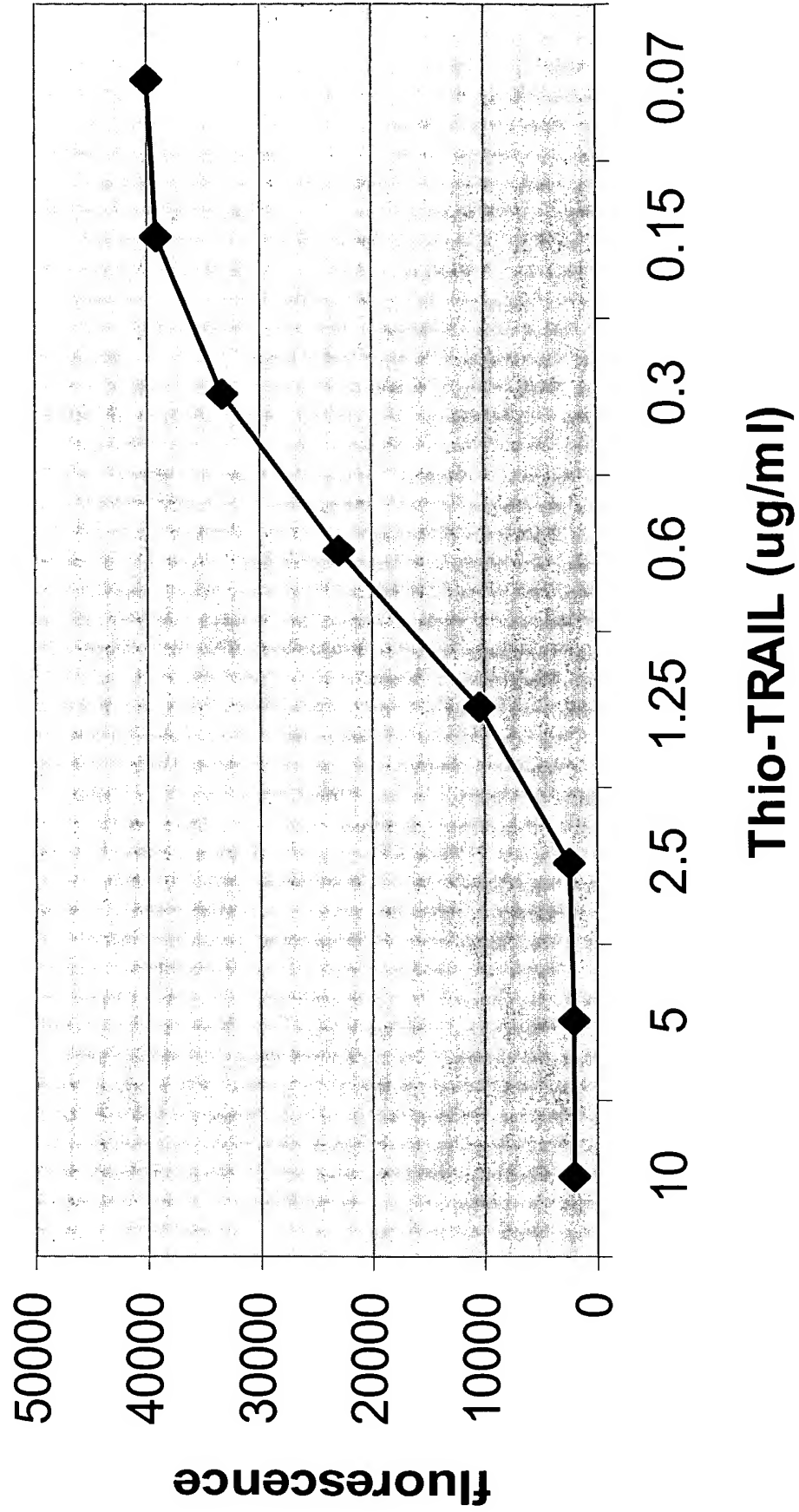
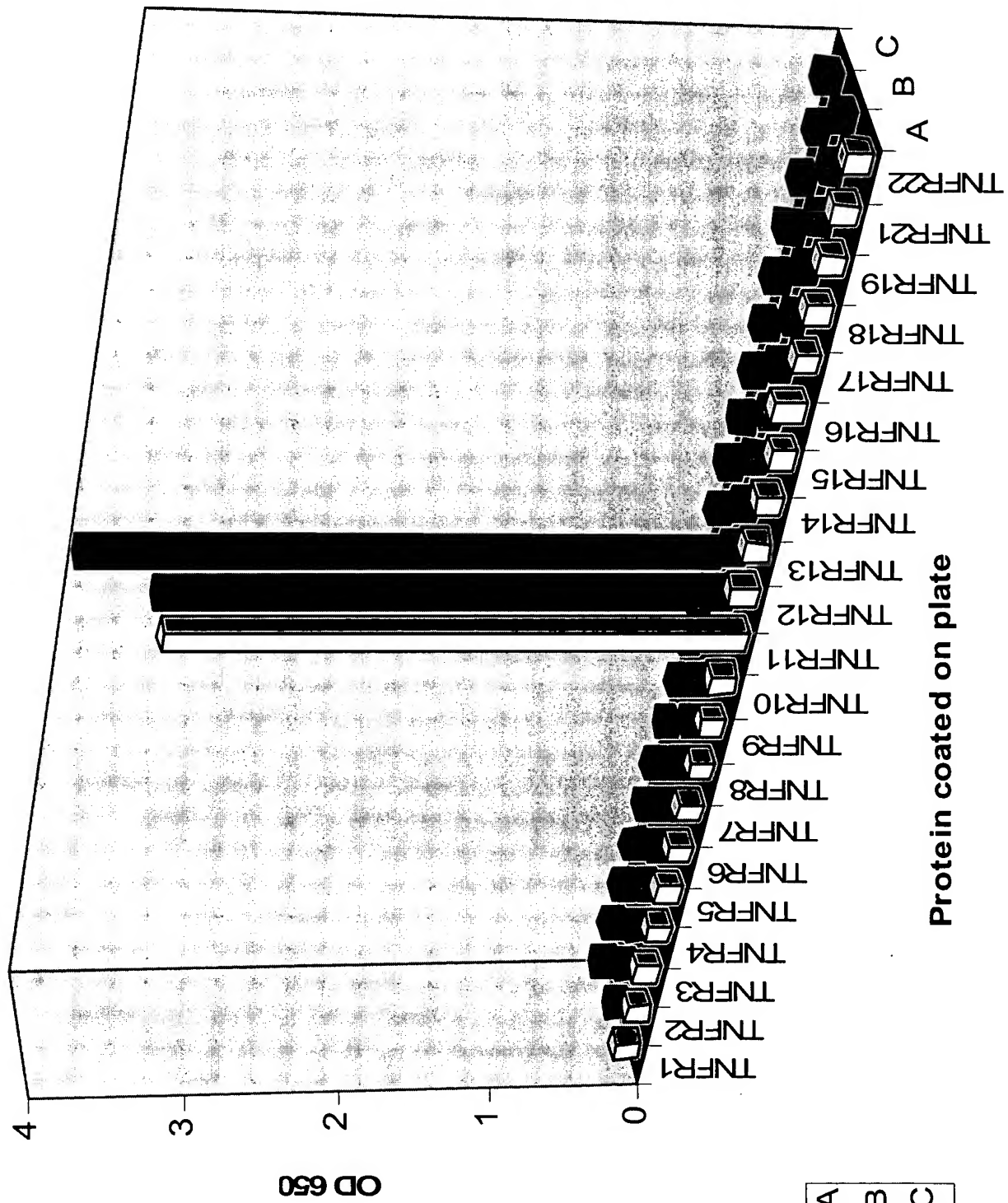


Figure 2

Specificity of DR5 Functional Antibodies



Effect of 3 Different DR5 Antibody Agonists On Jurkat Cells

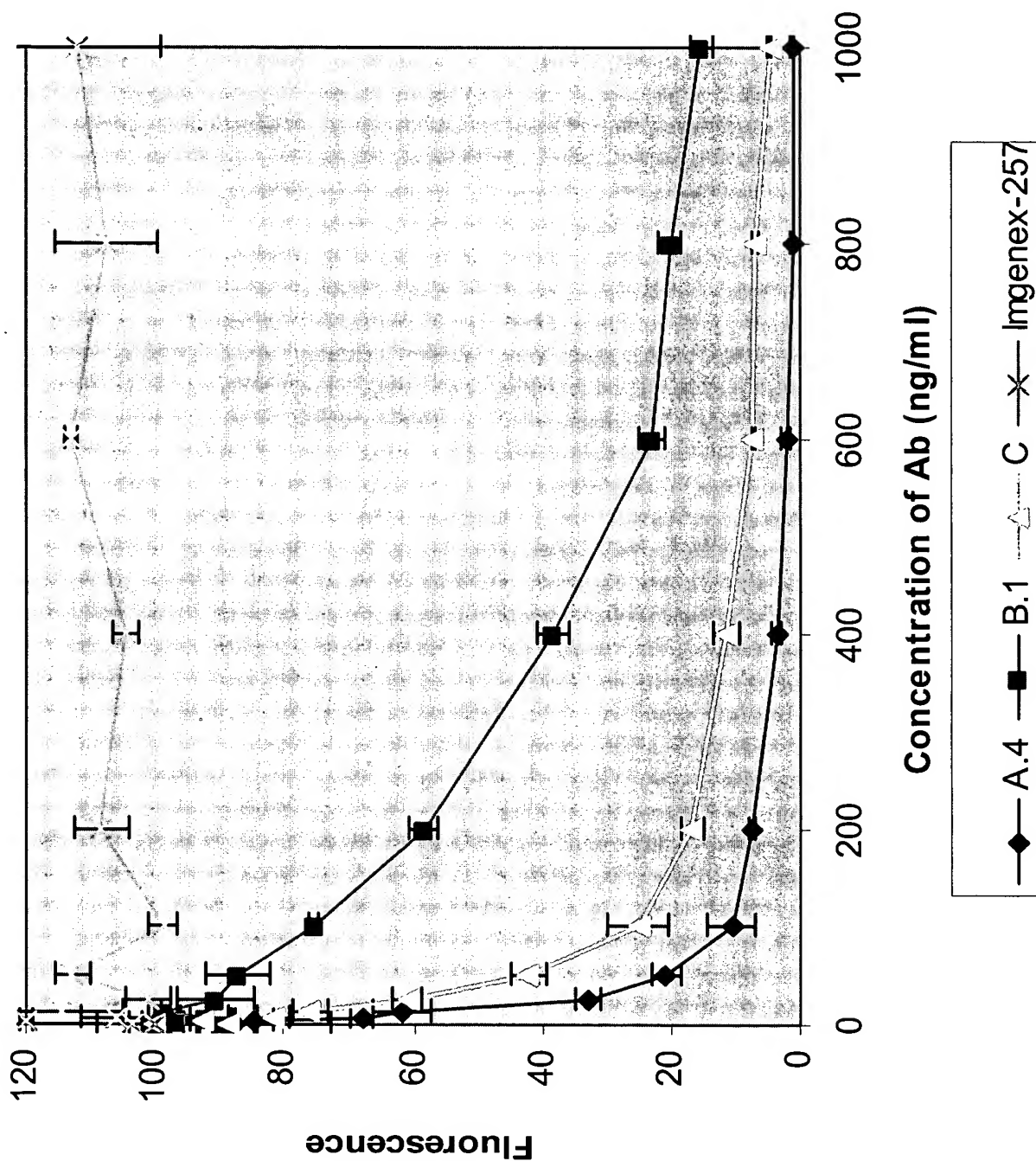


Figure 4

Caspase 3 activation in treated Jurkat cells

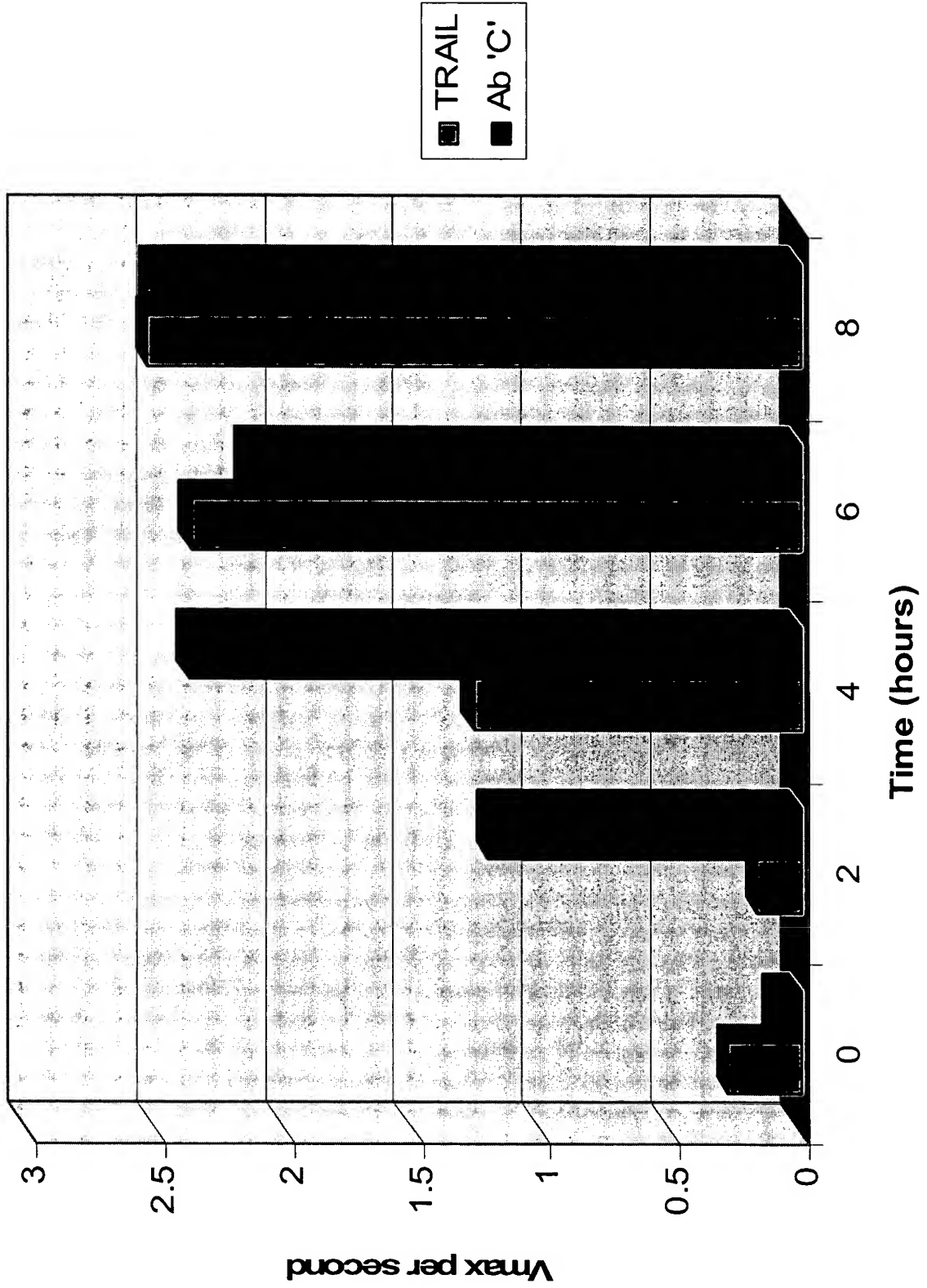
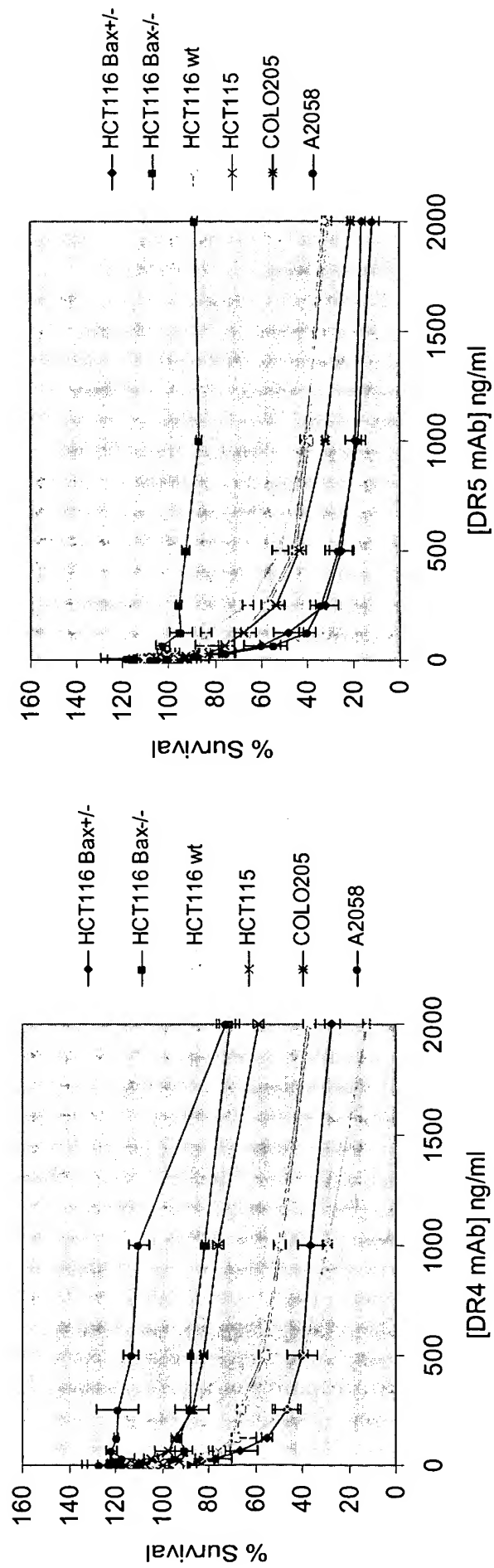
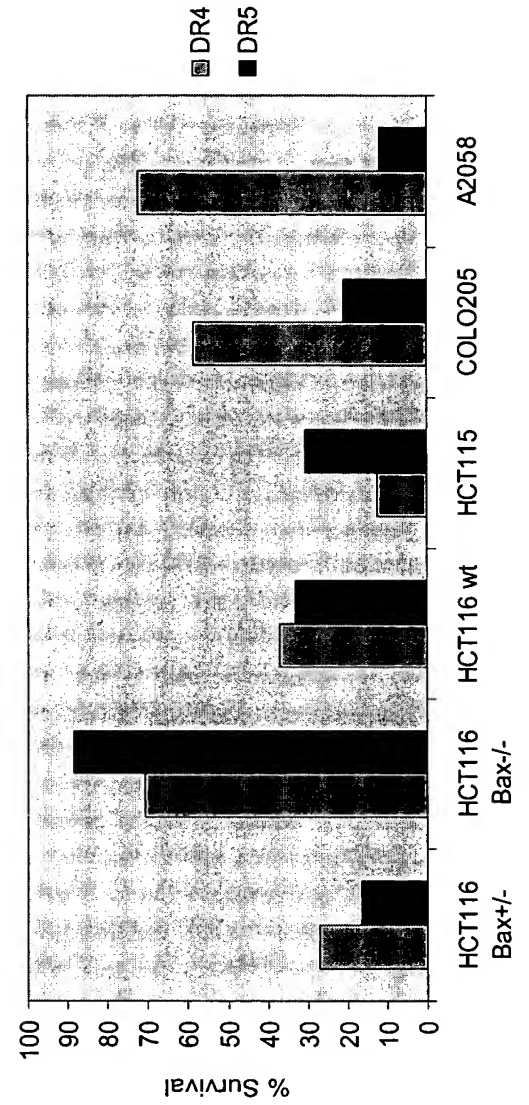


Figure 5

Effect of DR4 / DR5 Functional Antibodies on Colon and Melanoma Cancer Cell Lines



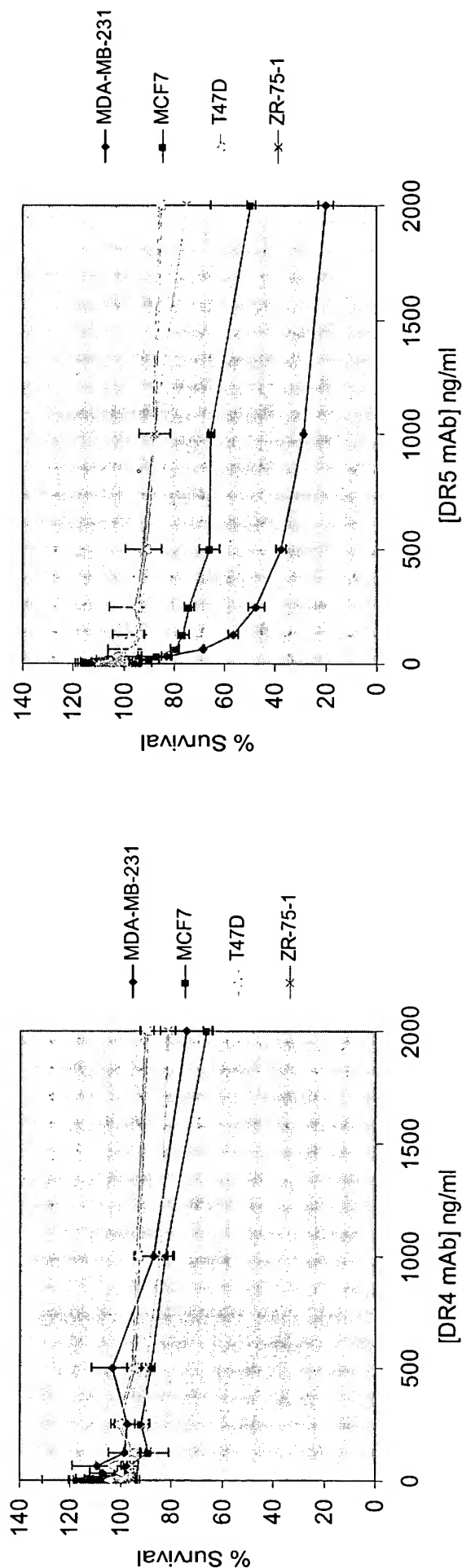
Effect of 2ug/ml Antibody on Cell lines



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Figure 6

Effect of DR4 / DR5 Functional Antibodies on Breast Cancer Cell Lines



Effect of 2ug/ml Antibody on various cell lines

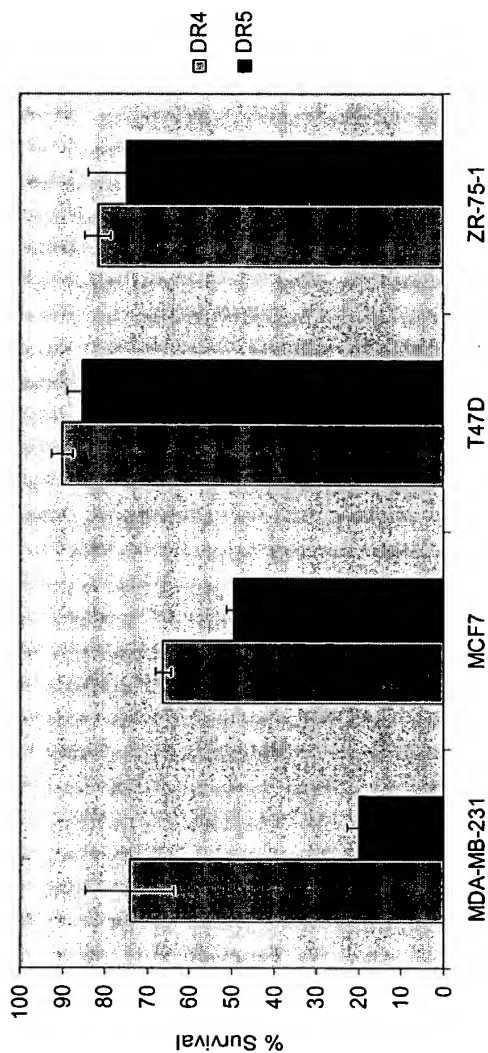


Figure 7

Dose Response To DR5 Antibody Agonist Normal vs Tumor Cells

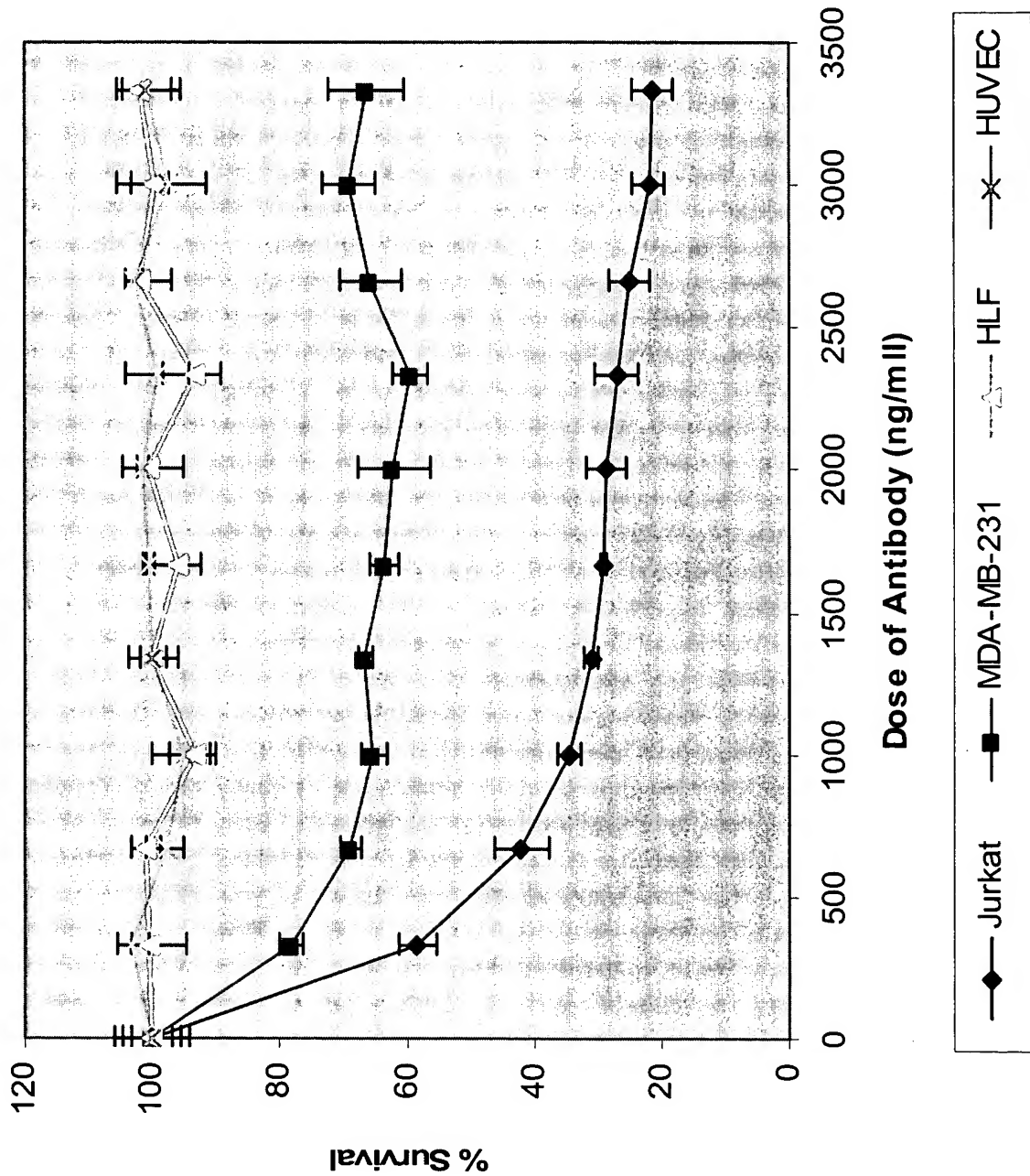


Figure 8

DR5 Antibody Agonist "A" - Caspase 3 Activation

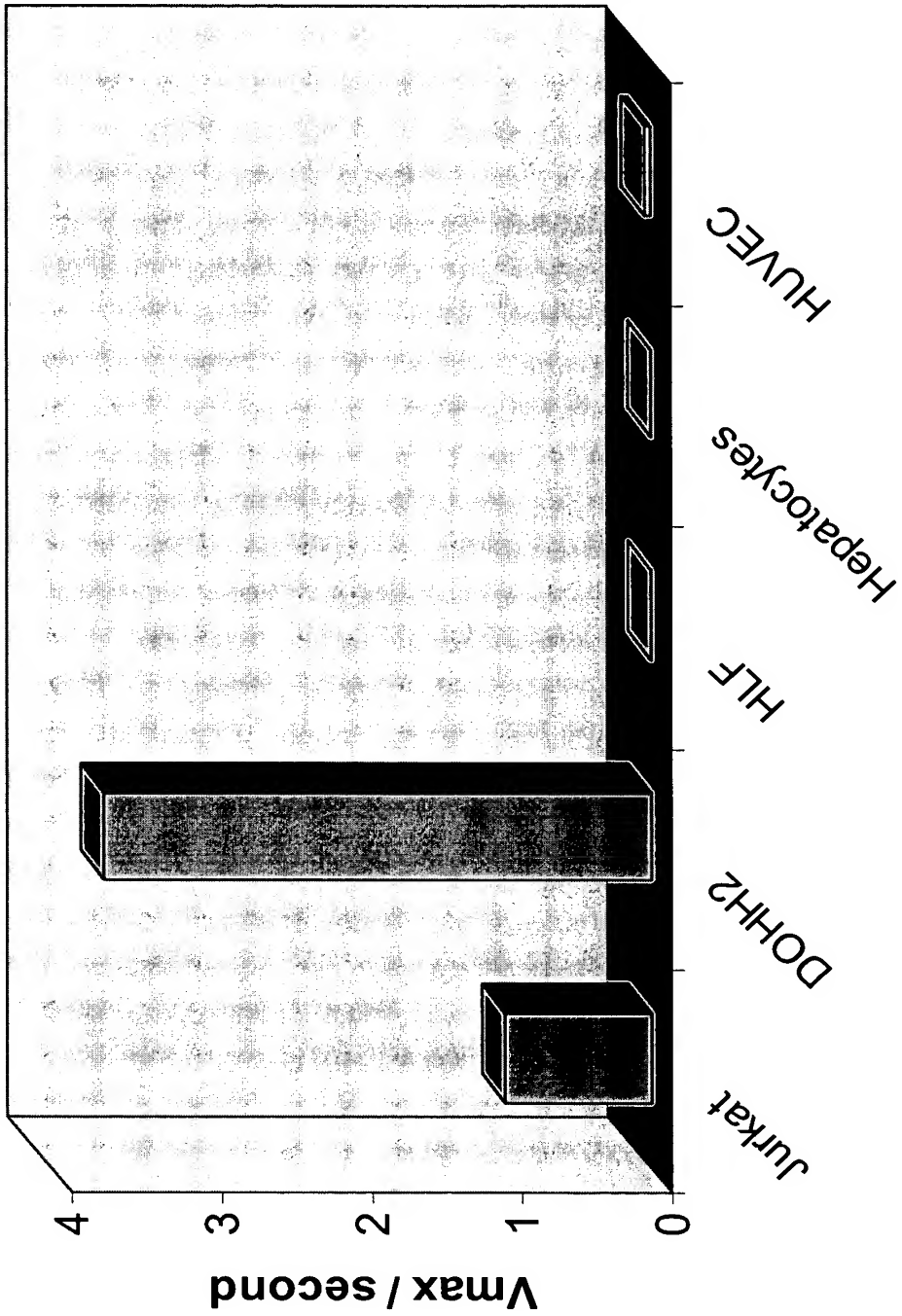


Figure 9

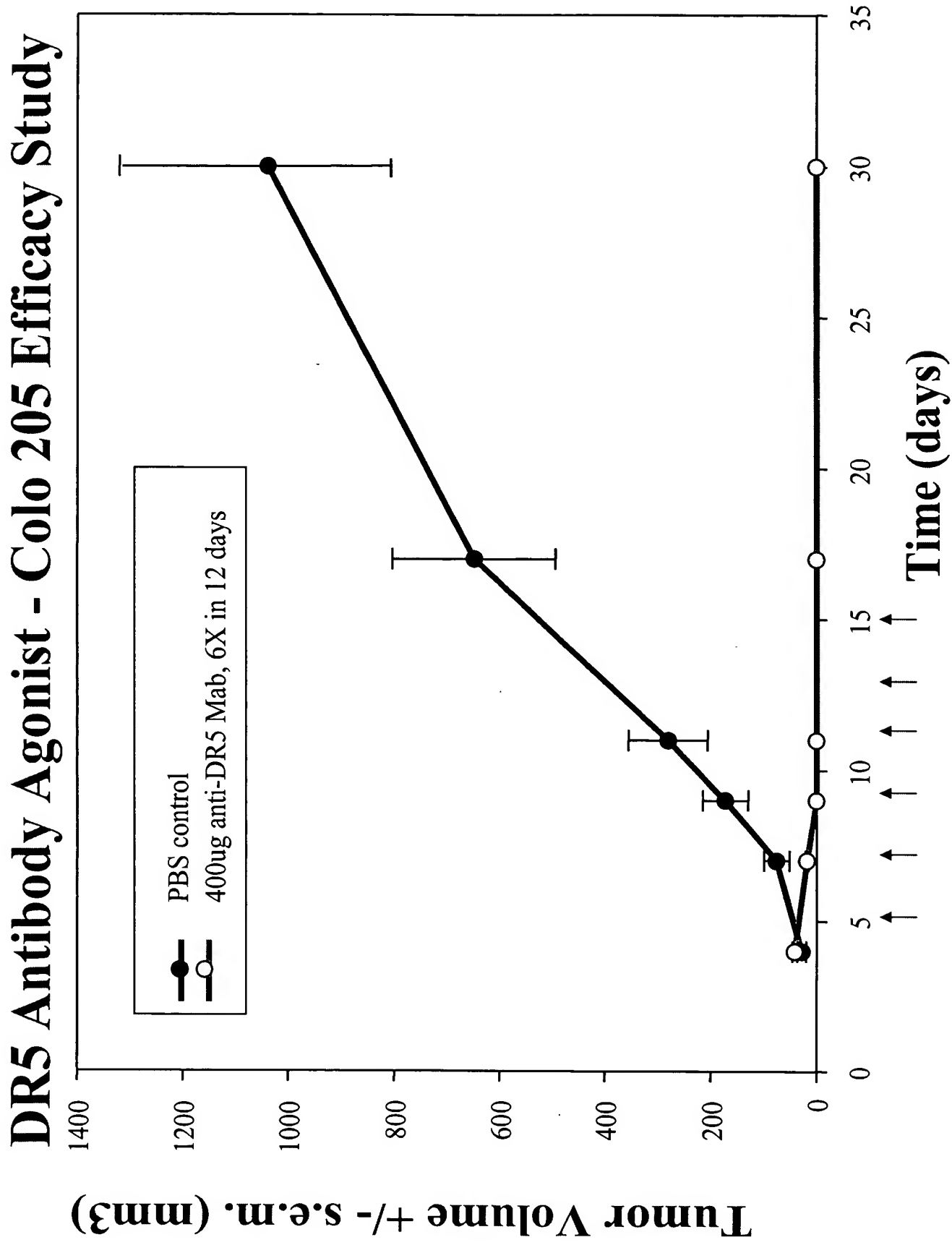


Figure 10

Anti-DR5 Dose Response, COLO205 Subcutaneous Model

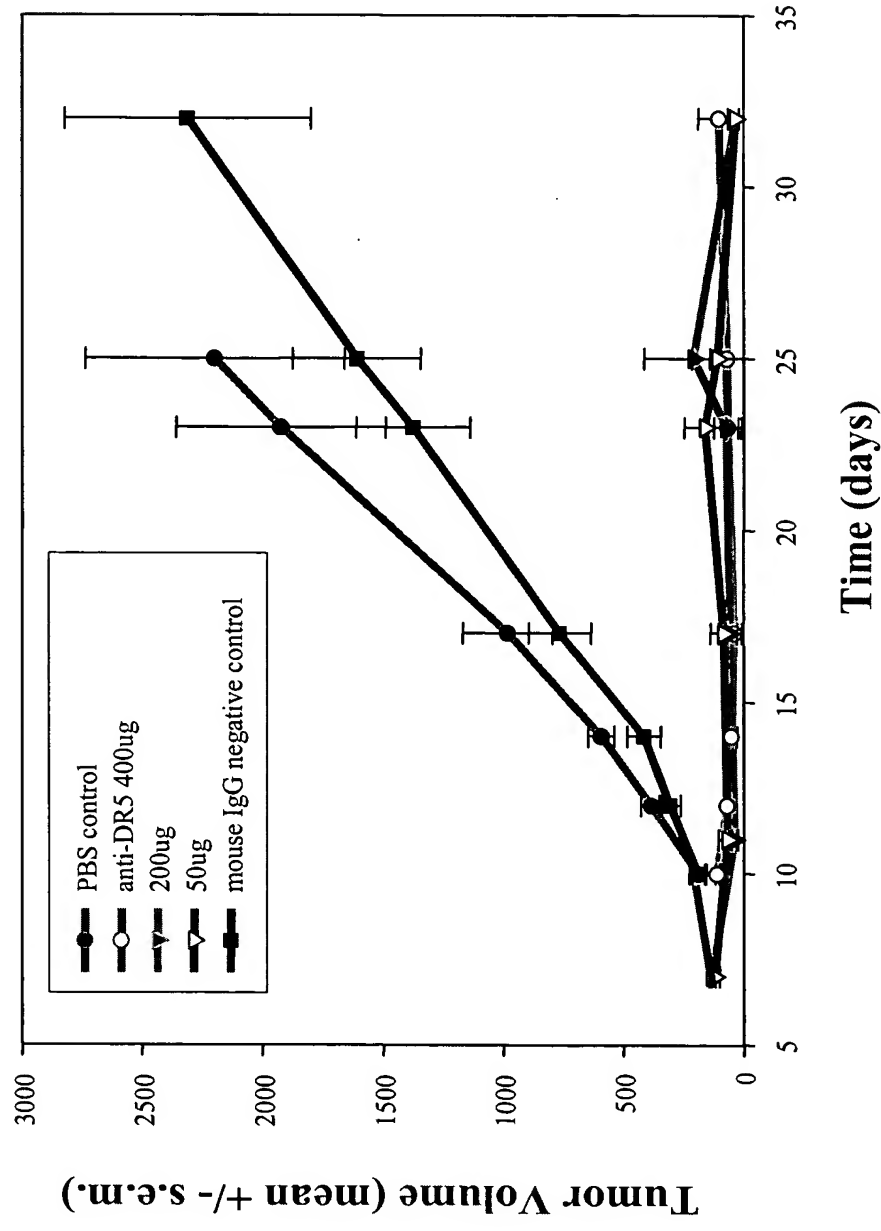
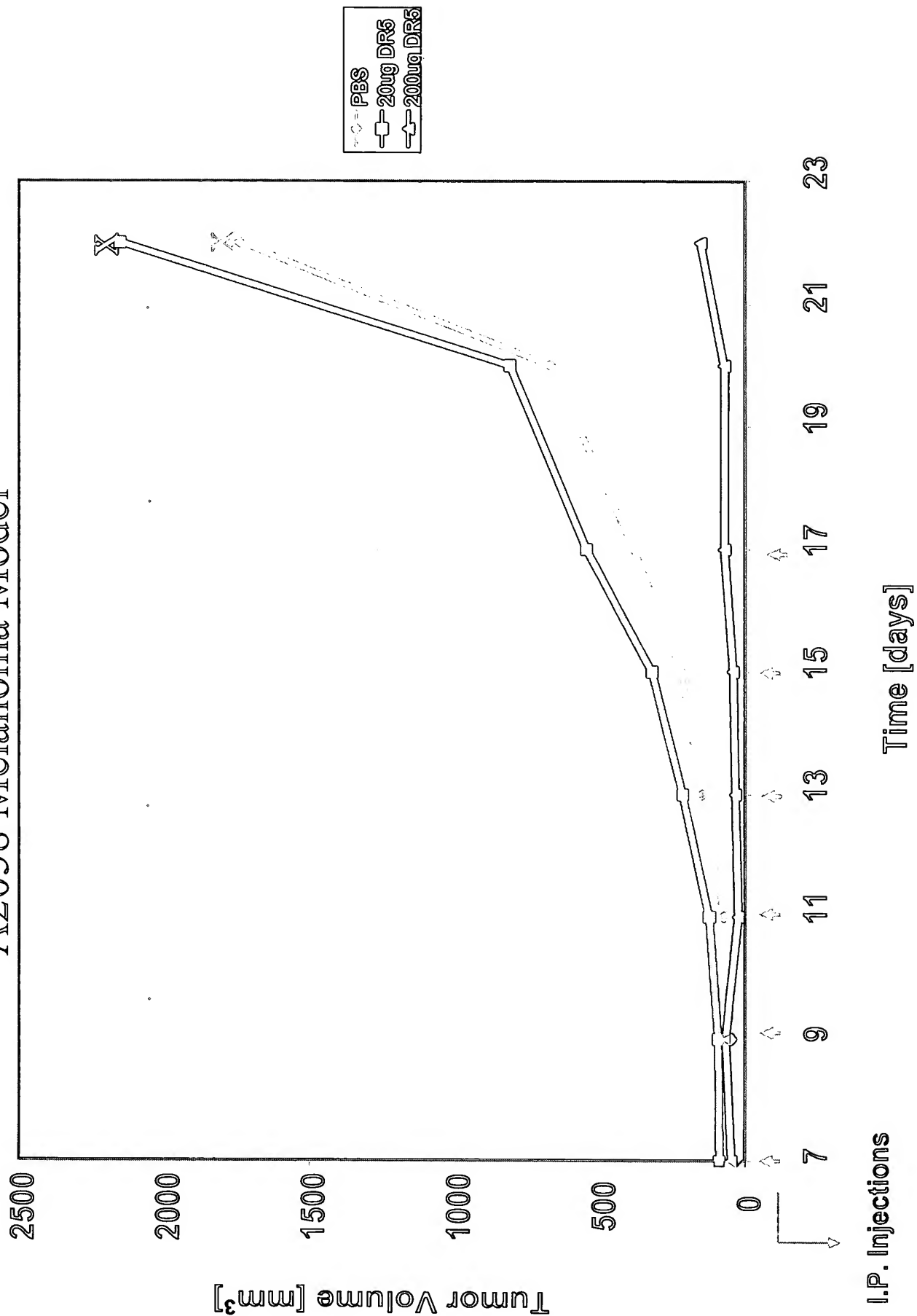
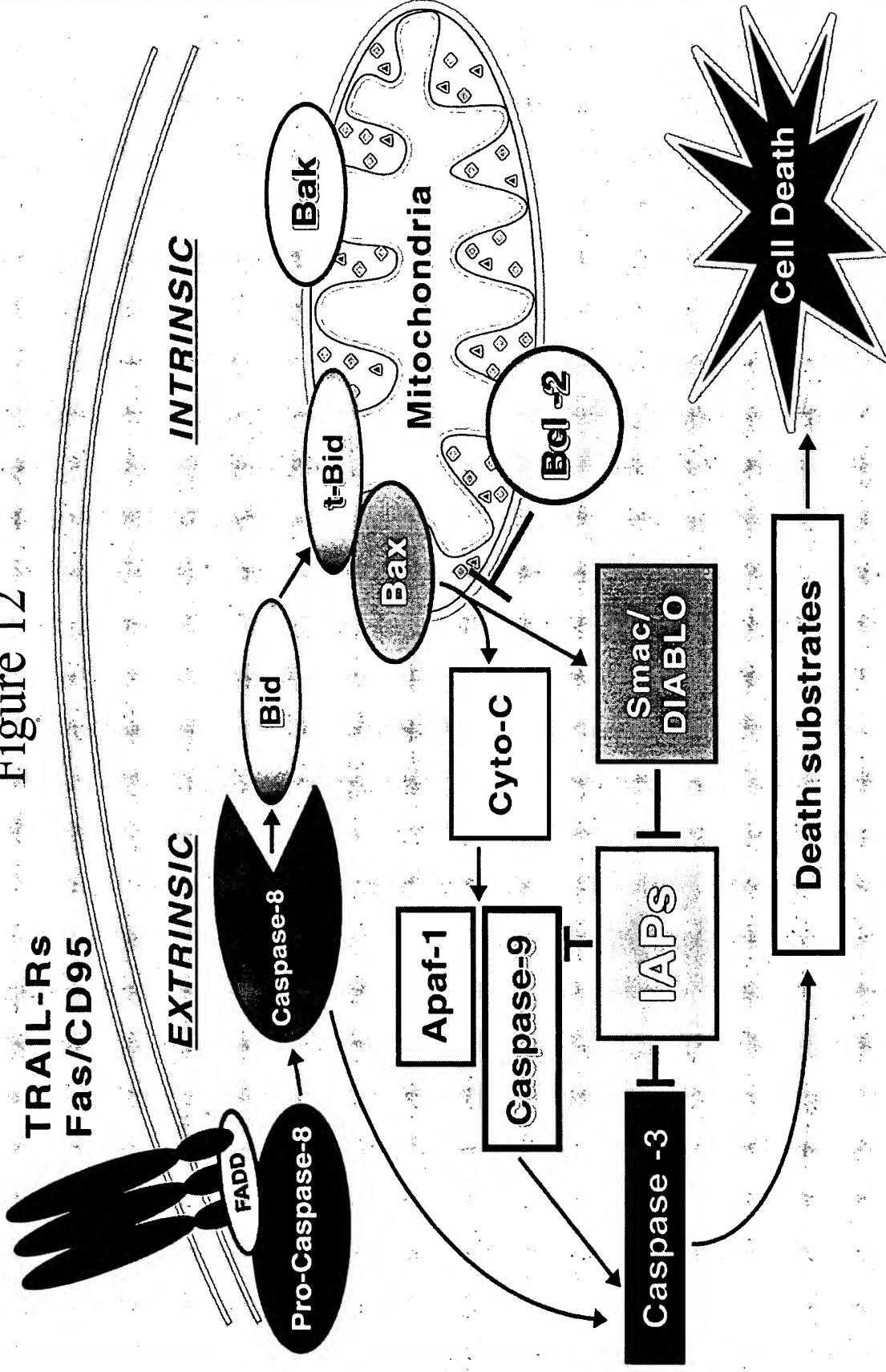


Figure 11
Tumoricidal Activity of DR5 Monoclonal Antibody In Vivo
A2058 Melanoma Model



Pathways For Caspase Activation and Apoptosis

Figure 12



Predominant Anti-Apoptotic Features of Tumor Cells:

- Over-expression of Bcl 2
- Increased Levels of IAP's
- Mutations in Bax

Anti-DR4 or DR5-induced apoptosis in A2058 cells in the absence or presence of 0.5 μ M LBP 672

Figure 13

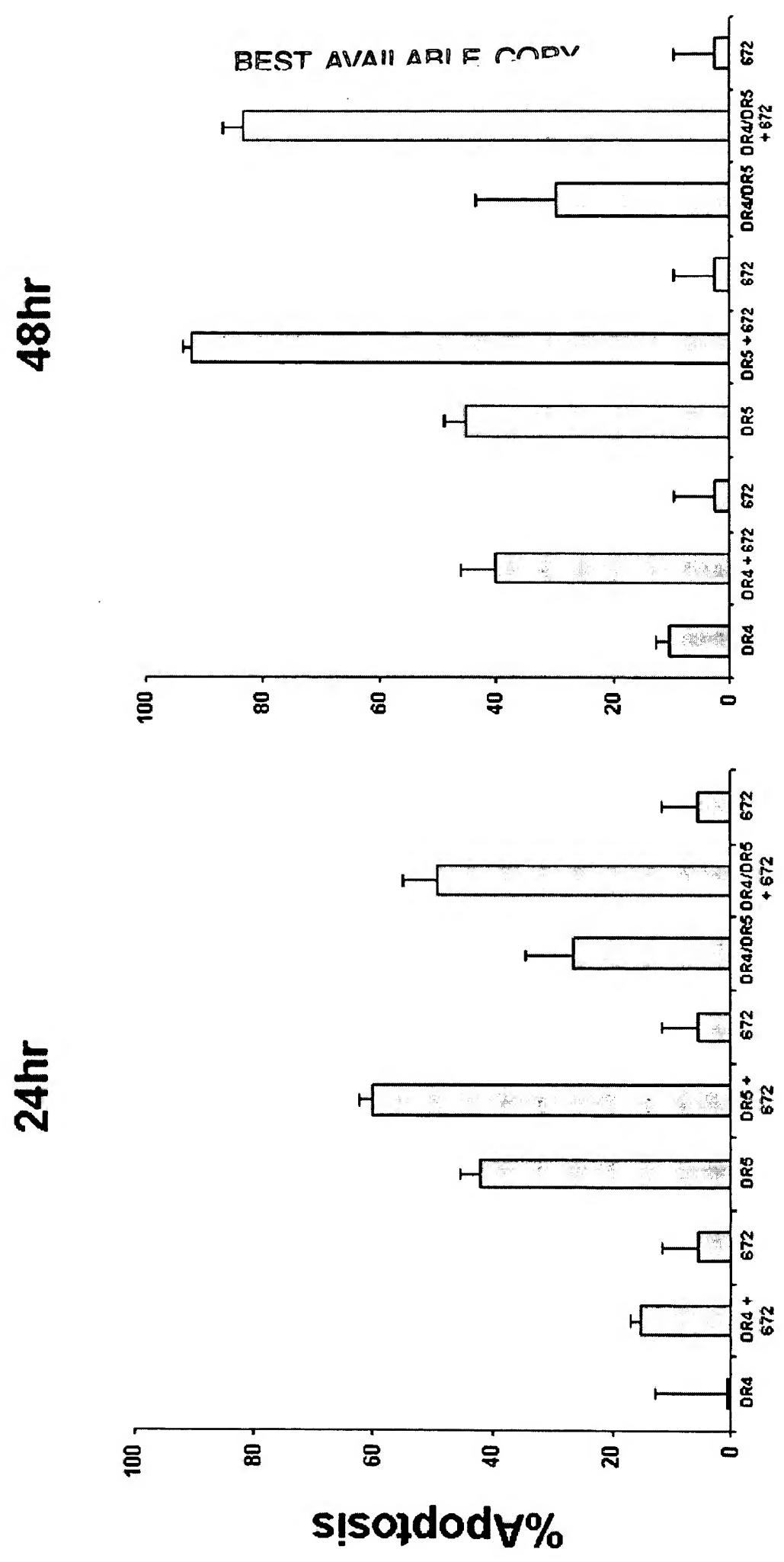


Figure 14

Effect of LB 672 On Normal And Tumor Cells

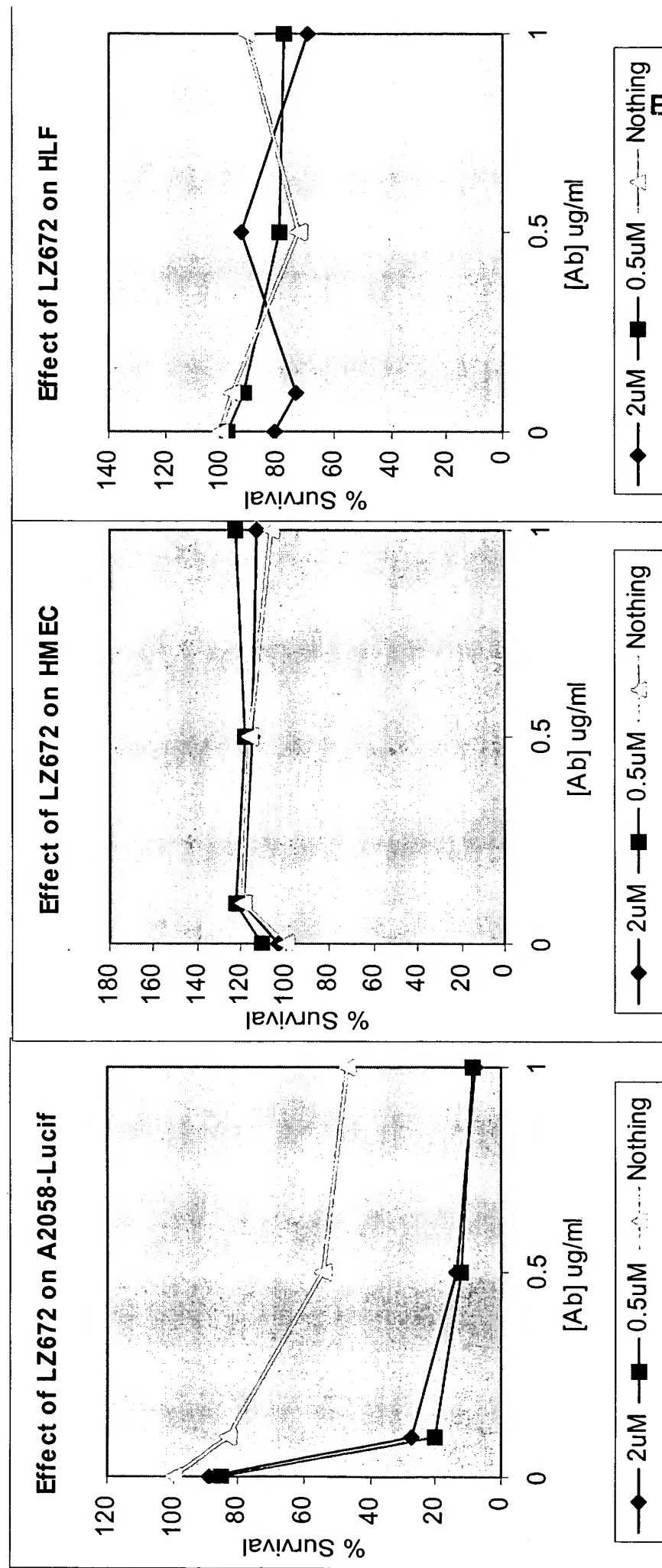
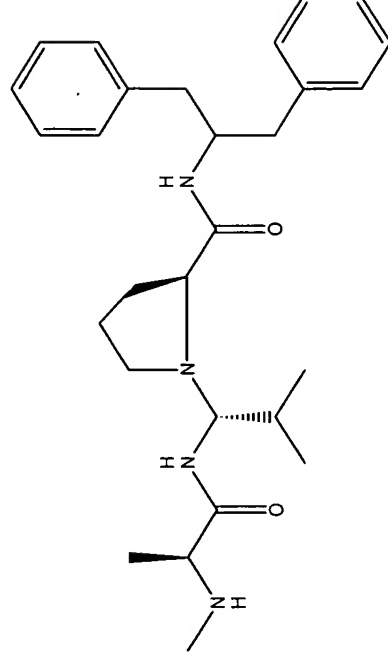
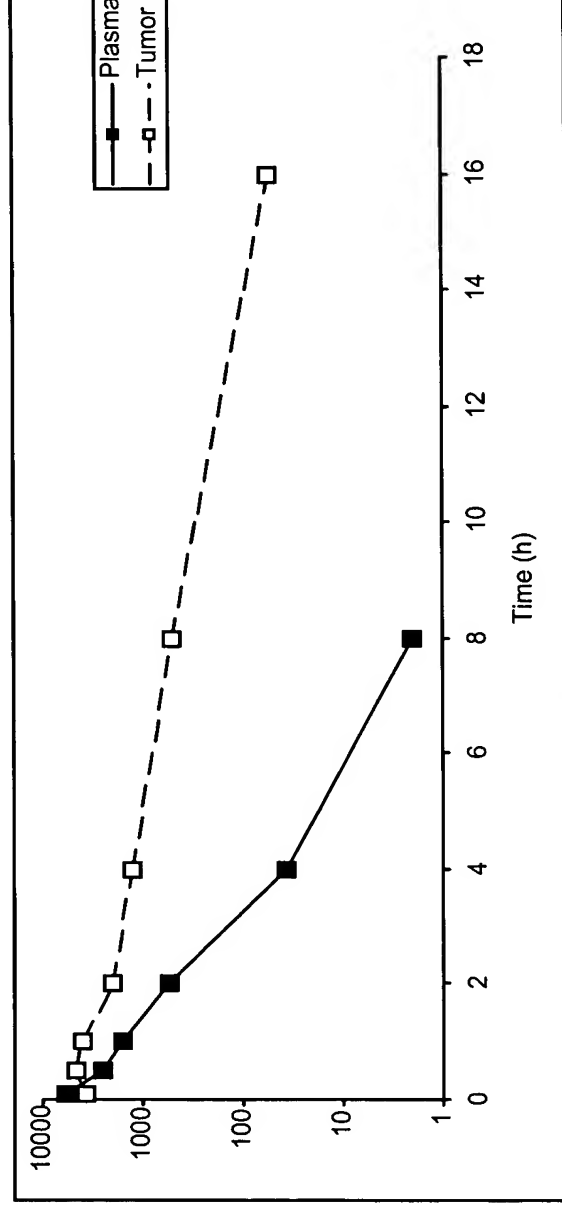


Figure 15

PK and PD Study of Smac Mimetic LBP672 In Nude Mice Bearing HCT116 Tumors



NVP-LBP672	
M.W. = 492.67 as a free base	

PK SUMMARY

Model: Athymic nude mice bearing subcutaneous HCT116 tumors

Dose: single 25 mg/kg i.v. trifluoroacetate salt (20.3 mg/kg free base) in D5W.

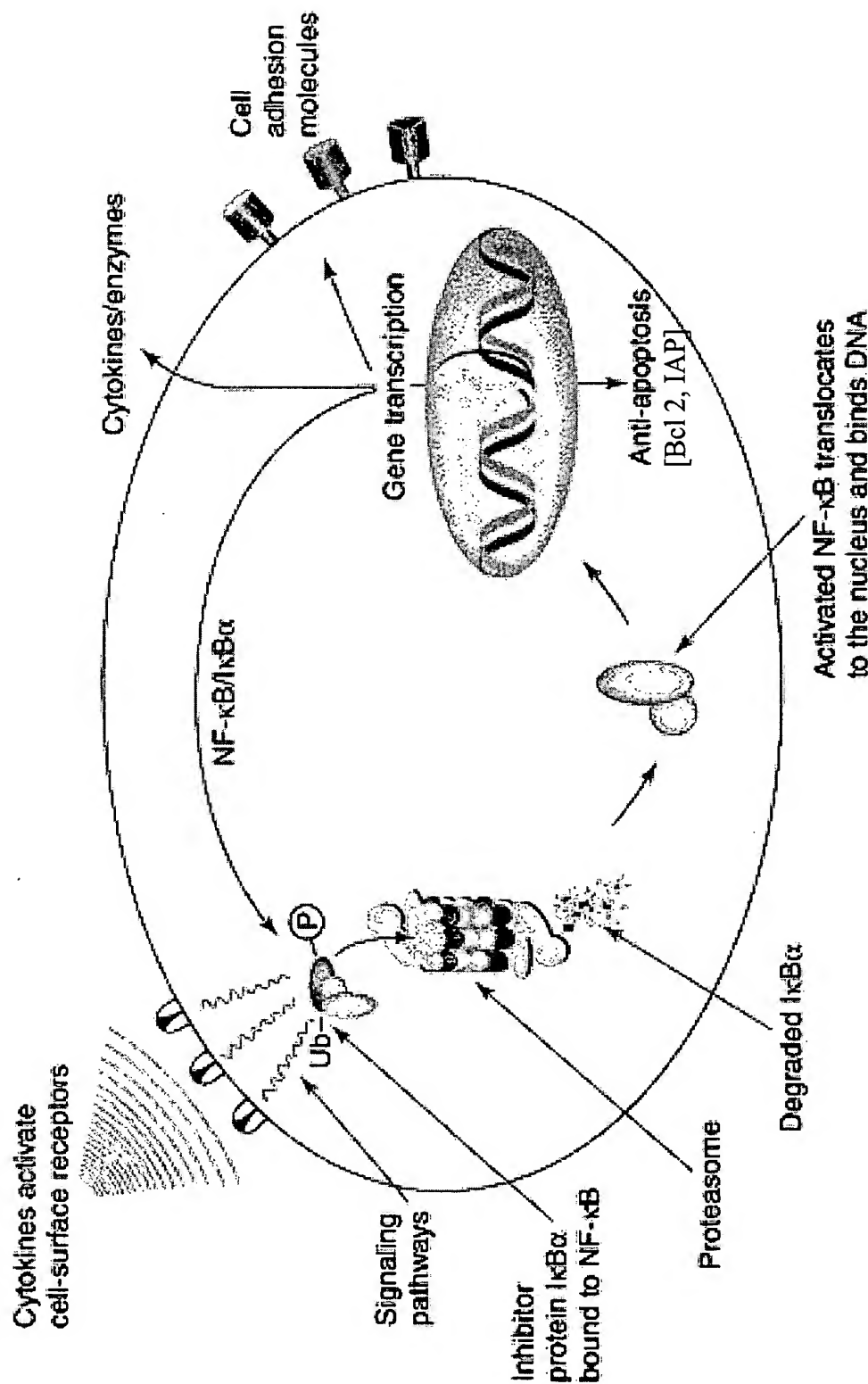
LBP672 rapidly absorbed by tumor Tmax @ 0.5 h. Mean tumor Cmax @ 4620ng/g (9.38 uM).

At 16 h. post dose LBP672 mean tumor [co] @ 55.1 ng/g (110 nM).

Figure 16

NFkB Activation By The Proteasome

Viruses, growth factors, radiation or chemotherapeutic drugs activate pathways that lead to the degradation of I κ B by the proteasome. NF κ B activates transcription of genes that protect the cell from apoptosis



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Proteasome Inhibitor MG132 Enhances DR5 Antibody Induced Apoptosis of SW 480 Human Colon Carcinoma Cells



Figure 18

20S Proteasome Inhibitors – [Chymotryptic Activity]

Compound	Description	Selectivity	IC50	MTD	Sol.
<i>NVP-AFB340-NX</i>	<i>Boronate</i>	<i>Good</i>	<i><1nM</i>	<i>3mg/kg-1x</i>	<i>Good</i>
<i>NVP-AFD314-NX</i>	<i>Boronate</i>	<i>Good</i>	<i><1nM</i>	<i>3mg/kg-1x</i>	<i>Good</i>
<i>NVP-AEV273-NX</i>	<i>Beta-Lactame</i>	<i>Excellent</i>	<i>3nM</i>	<i>20 mg/kg-2x</i>	<i>Poor</i>

NVP-AFB340-NX

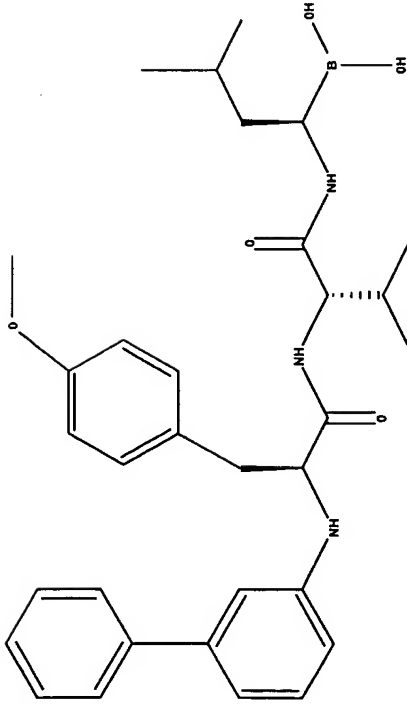


Figure 19
Effect of Proteasome Inhibitors On A2058 -Luc

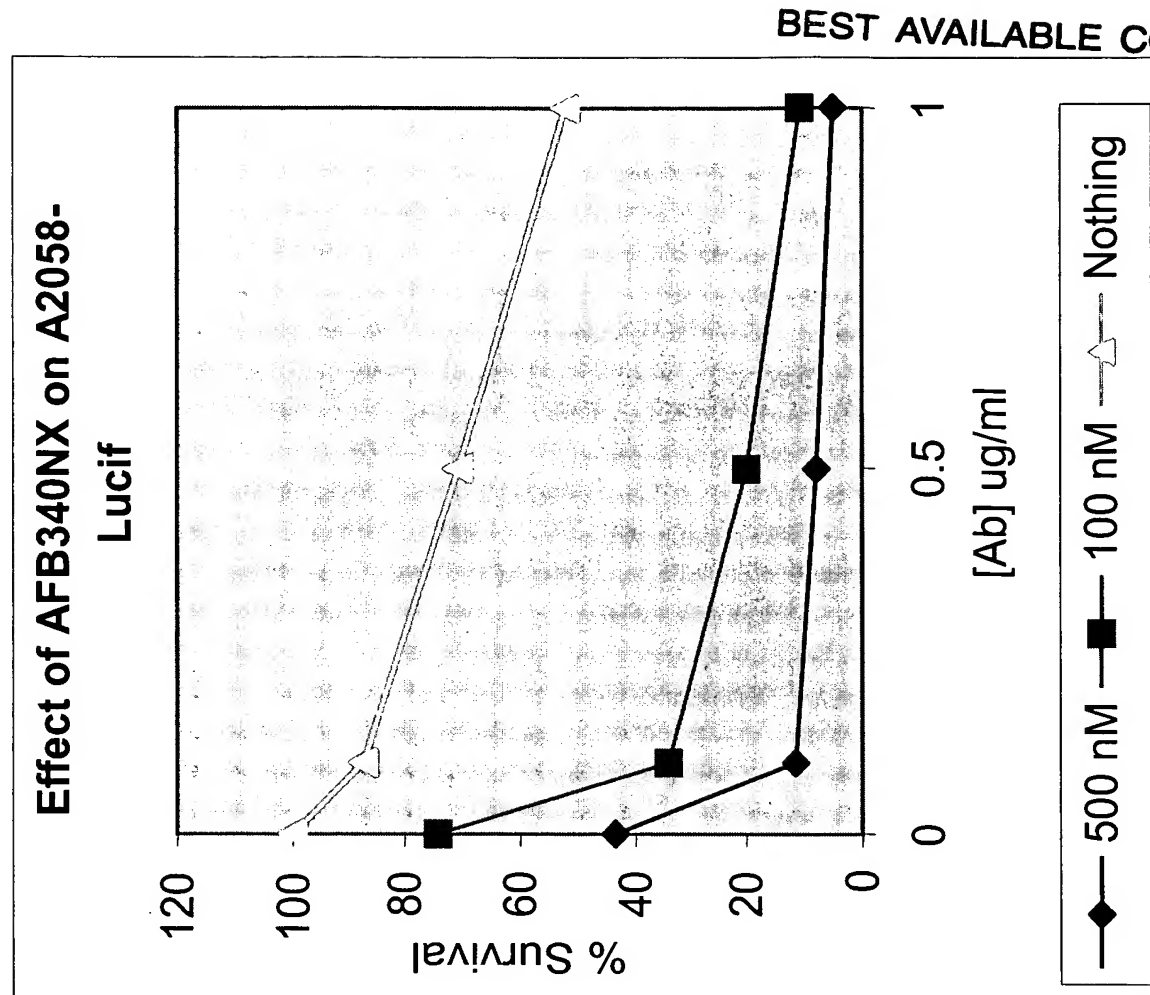
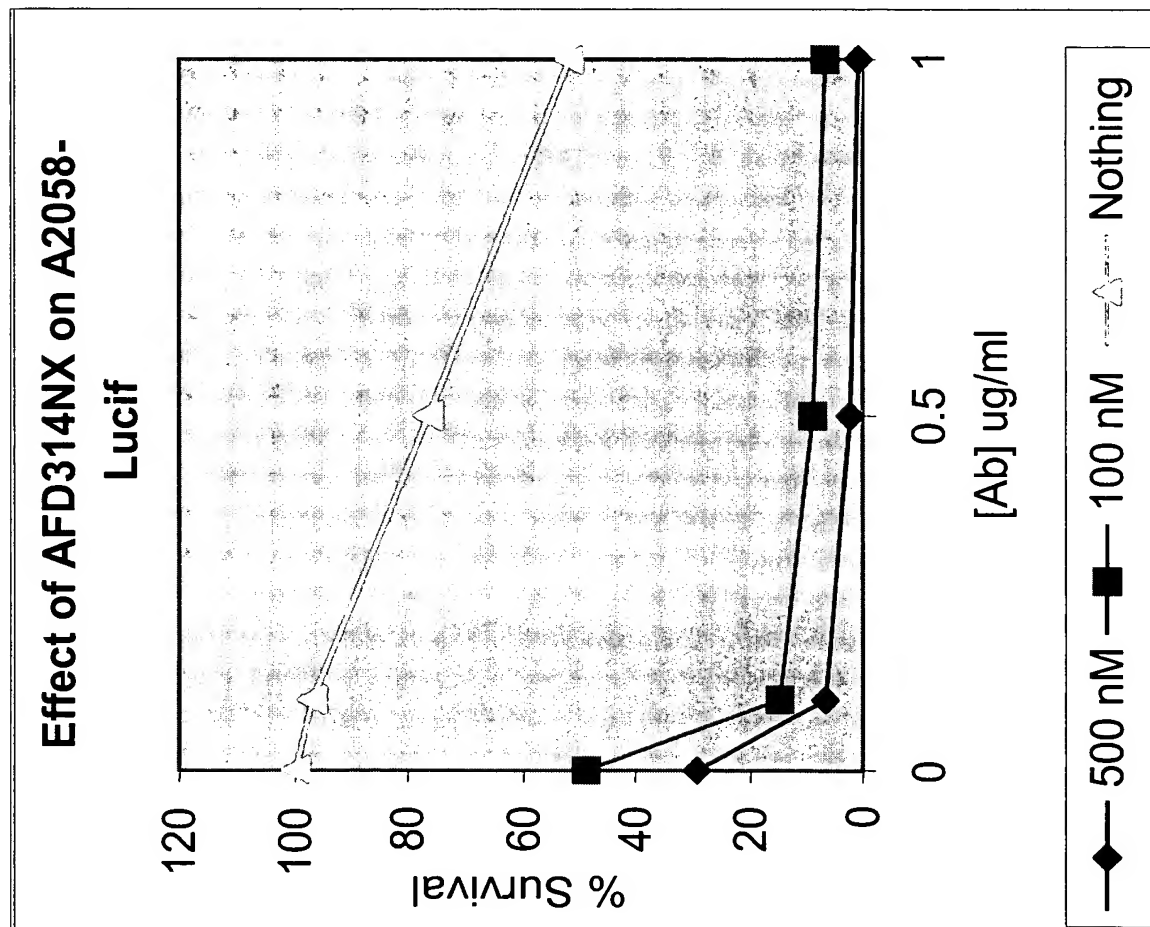


Figure 20

Effect of Proteasome Inhibitors On The Hepatocarcinoma

Cell Line HUH7-Luc

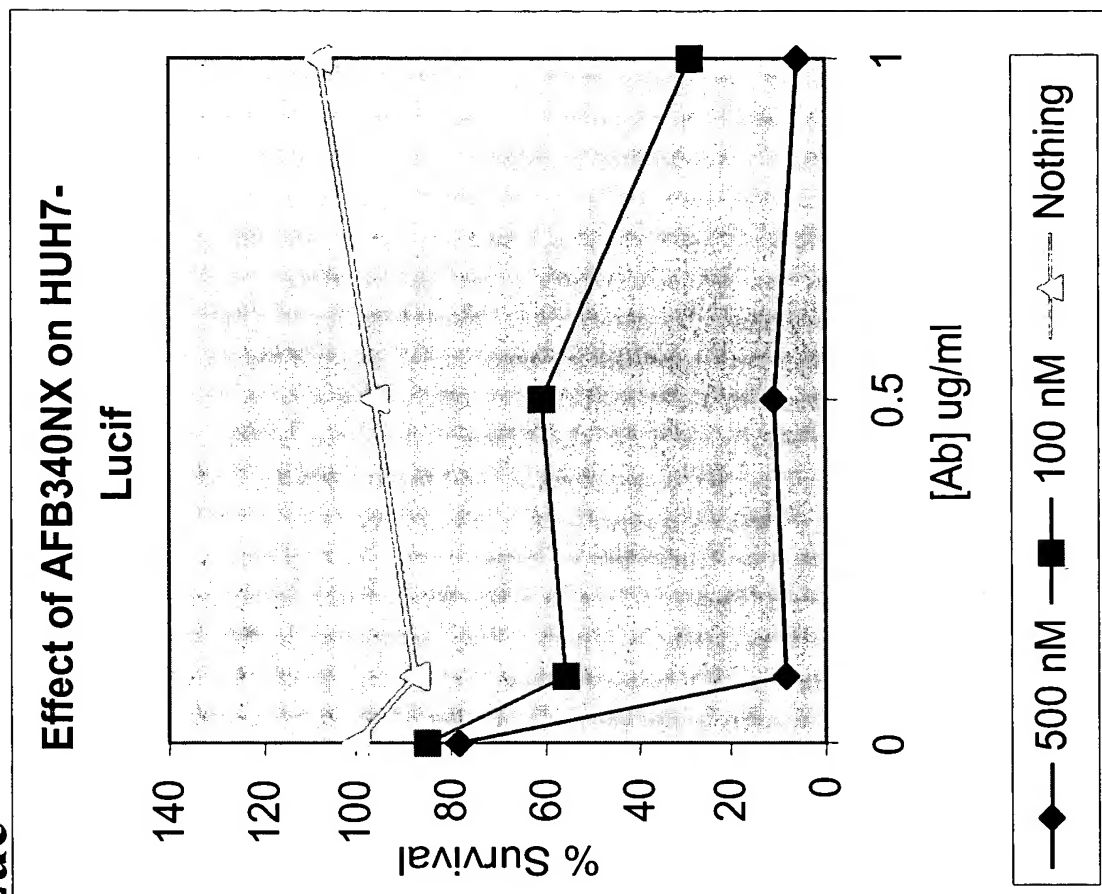
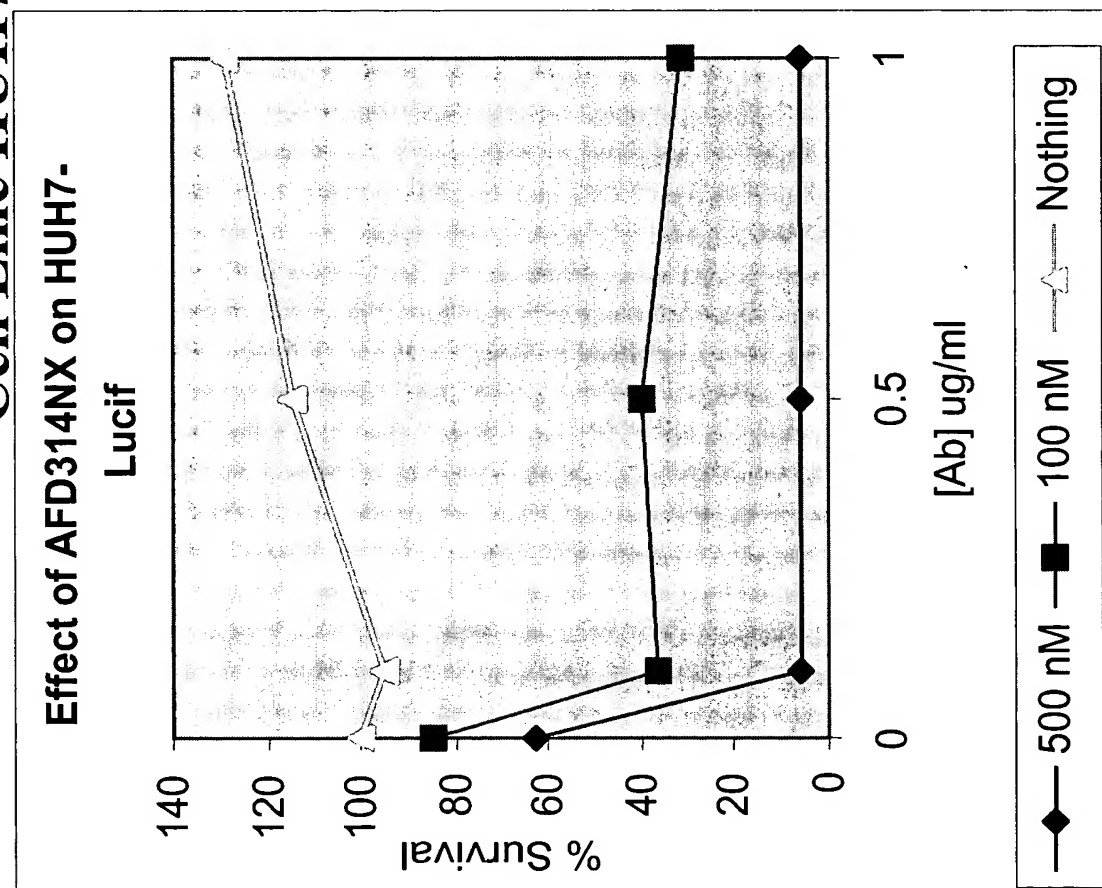


Figure 21

Effect of Proteasome Inhibitors On Normal Human Mammary Epithelial Cells (HMEC)

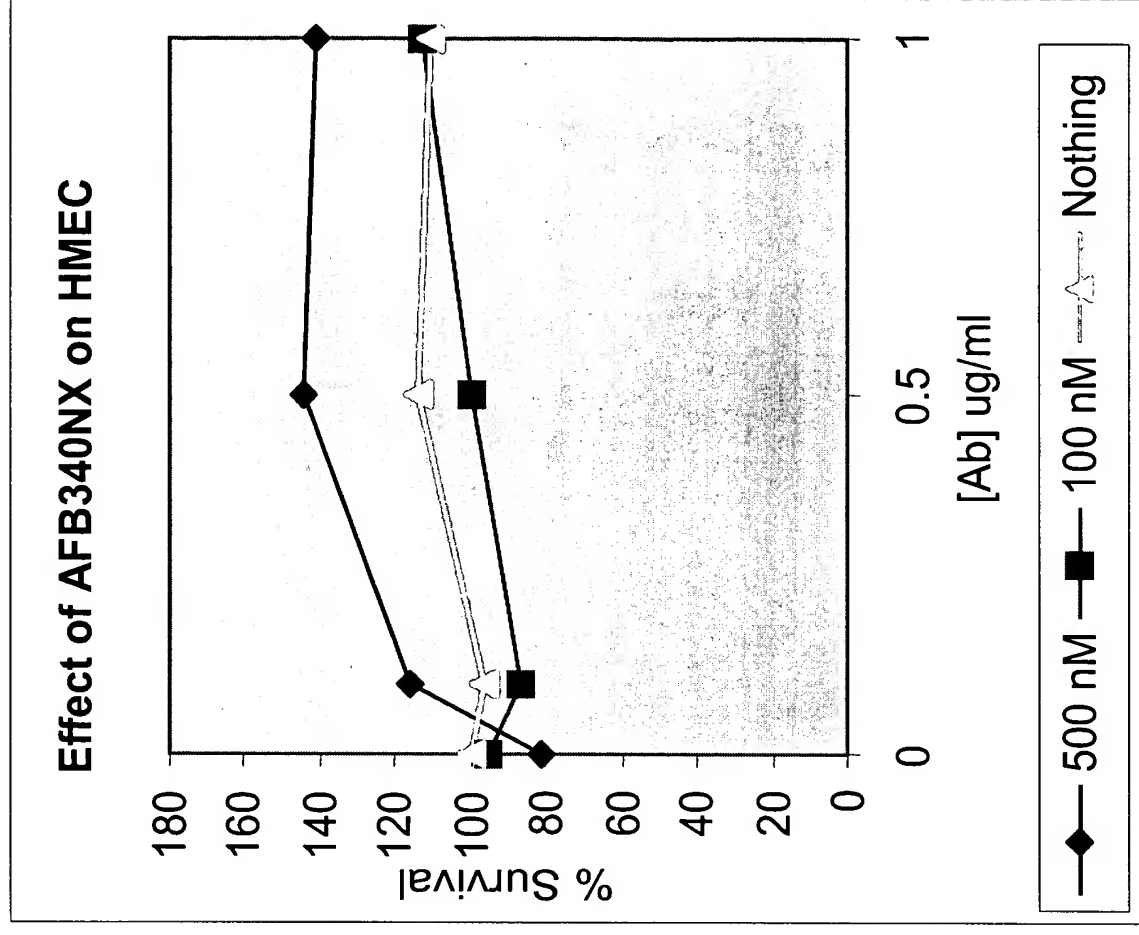
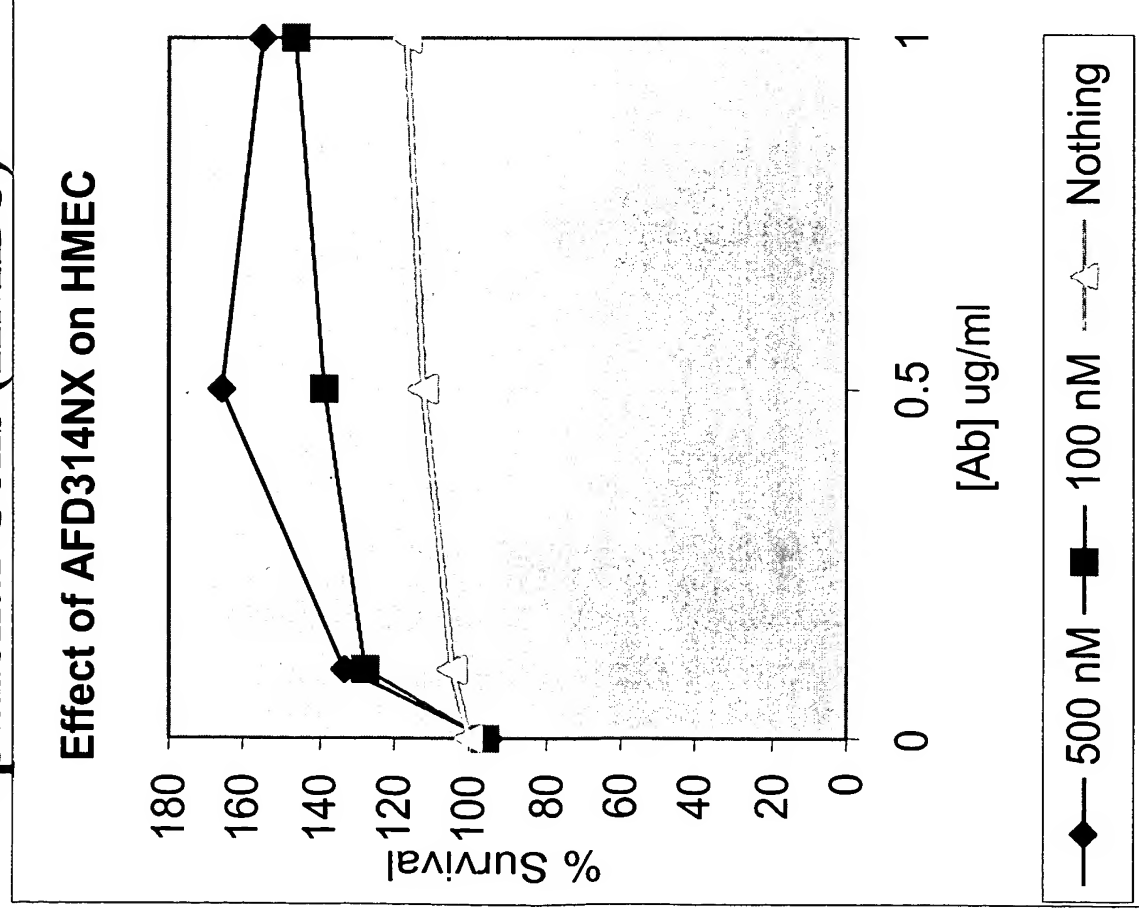


Figure 22

Functional Activity

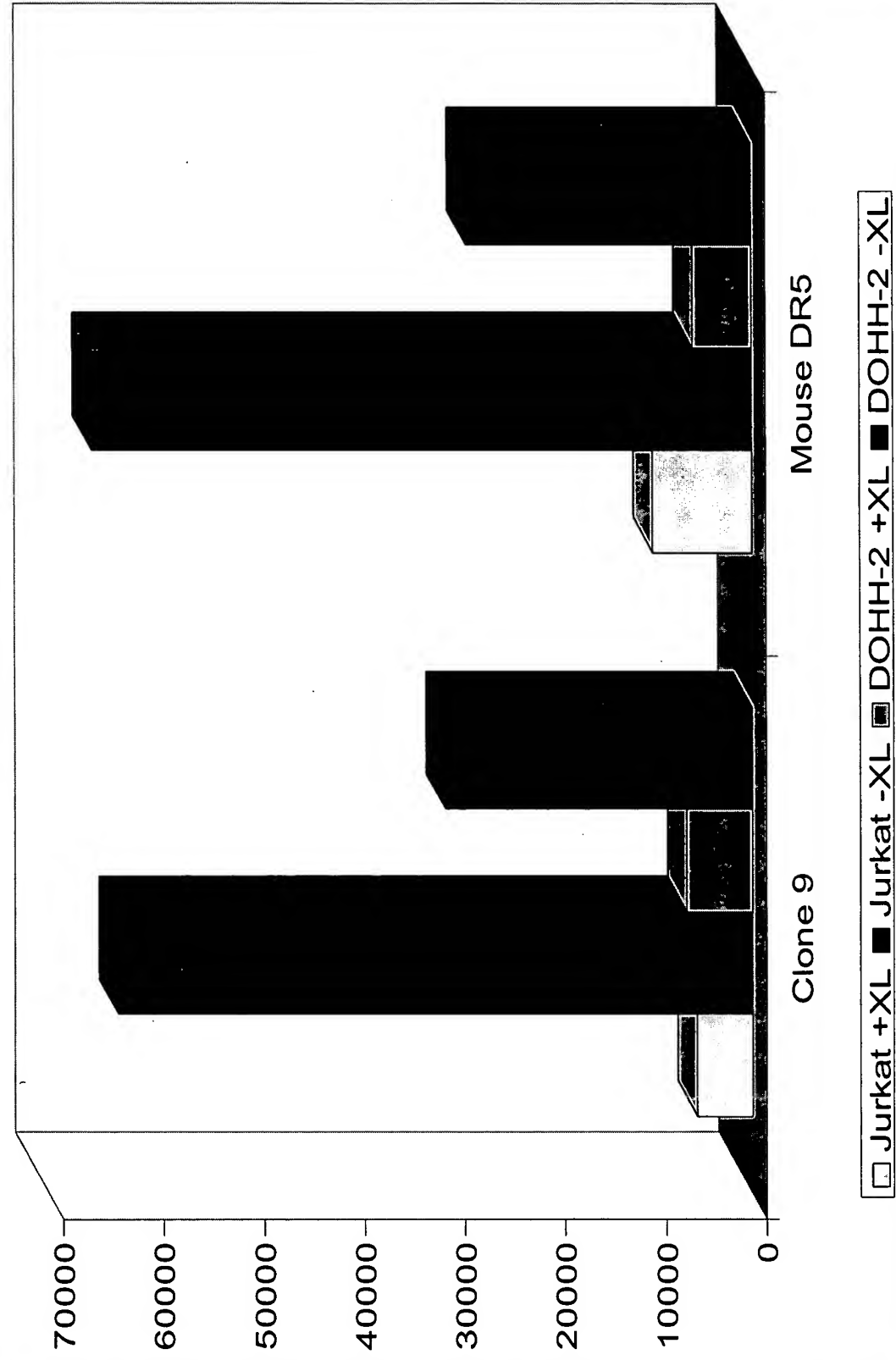


Figure 23

Anti-DR5 DNA Sequence

Light Chain Variable Region

GACATTGCGATGACCCAGTCTCACAAAGTTTCATGTCCACATTAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAATAA
ACCAGGGCAATCTCCTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTA
TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGAGTAGTAACCCGCTCAC
GTTCCGGTGTGGACCAAGCTGGAGCTGAACGGGCTGATGCTGCACCAACTGTATCC
ATCTTCCCACC

Heavy Chain Variable Region

CAGGCAAAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAAACCCGGGCATCA
GTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACTAGGGT
AAAGCAGAGGTCTGGACAGGGTCTTGAGTGGAATGGGTGTTTACCCTGGAGGTGGT
TATATAAAATACAAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCT
CCAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGGCTCTGCGGTCTATTTC
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTCA
CAGTCTCCTCA

Figure 24

DR5 V_H Sequence – Heavy Chain Subgroup 2B

FR1

Lys Val Gln Leu Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val

CDR1

Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Thr Ile His Trp Val

FR2

Lys Gln Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Gly

CDR2

Gly Tyr Ile Lys Tyr Asn Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ala Asp

FR3

Lys Ser Ser Asn Thr Val Tyr Met Glu Leu Ser Arg Leu Thr Ser Glu Gly Ser

CDR3

Ala Val Tyr Phe Cys Ala Arg His Glu Glu Gly Ile Tyr Phe Asp Tyr Trp Gly

FR4

Gln Gly Thr Thr Leu Thr Val Ser Ser

Figure 25

DR5 V_L Sequence– Kappa Light Chain Subgroup 5

FR1

Asp Ile Ala Met Thr Gln Ser His Lys Phe Met Ser Thr Leu Val Gly Asp

CDR1

Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Ile Ala

FR2

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Ile Tyr Trp Ala

CDR2

FR3

Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly

Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr

CDR3

FR4

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr

Lys Leu Glu Leu Lys Arg Ala

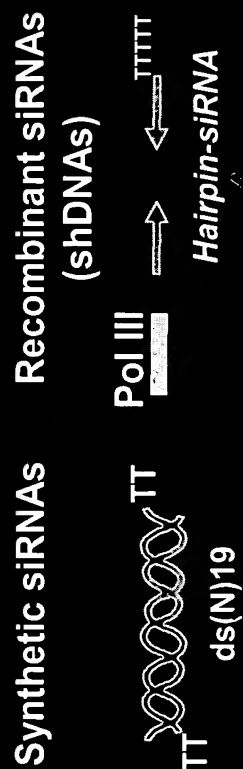
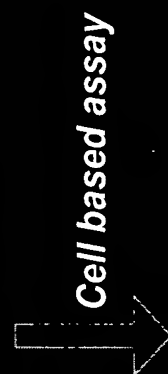
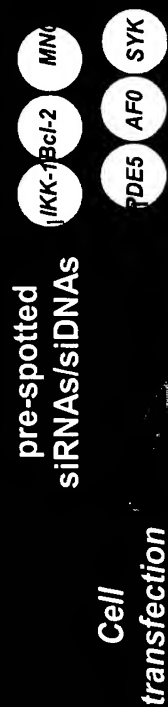


Figure 26



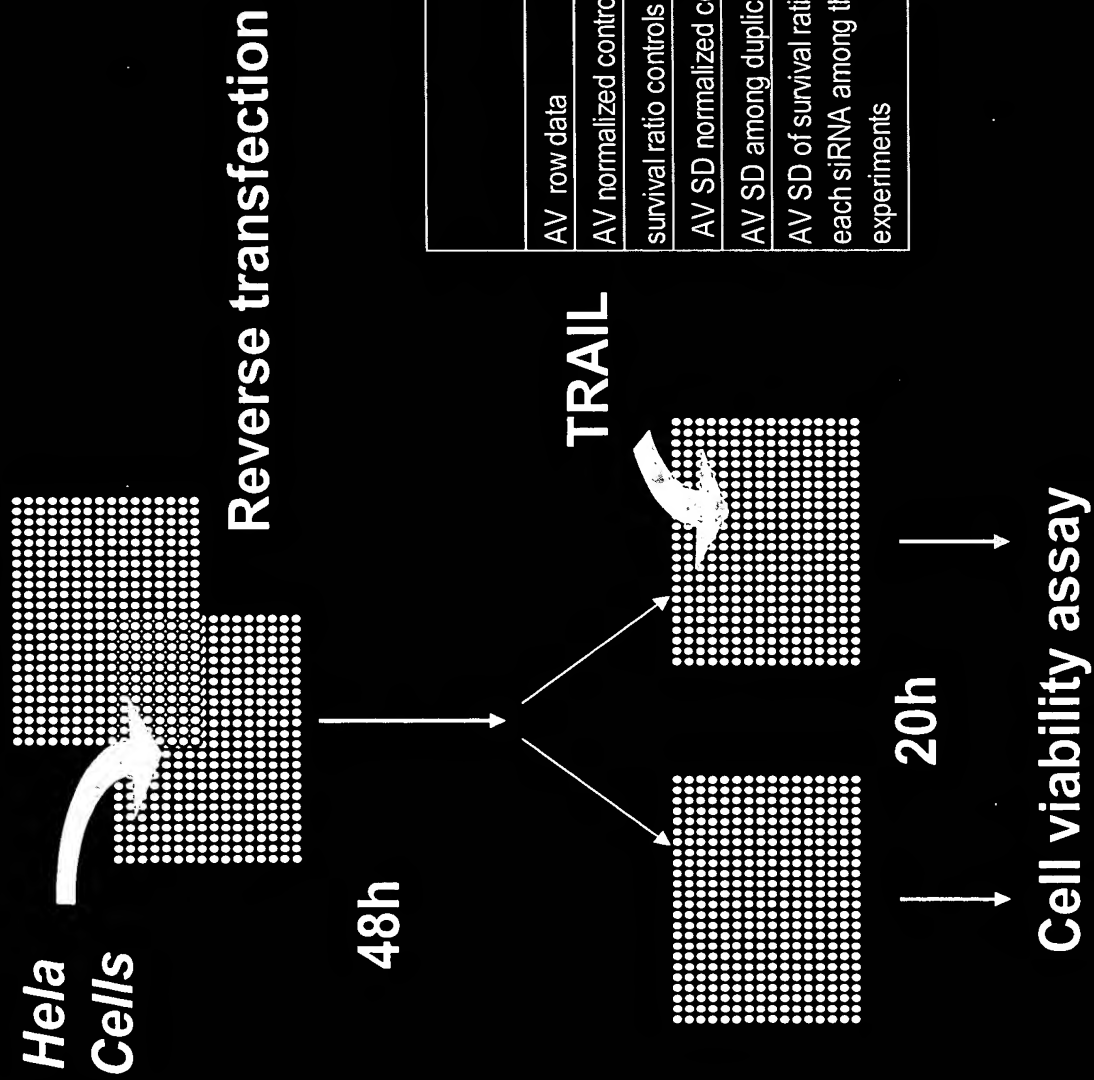
Assays:

Viability, cell survival

Reporter assays

Image based

Figure 27
TRAIL induced apoptosis in Hela cells



	screen 1		screen2	
	(-TRAIL)	(+TRAIL)	(-TRAIL)	(+TRAIL)
AV row data	29221.7	9368.75	59419.2	26782.7
AV normalized controls	100	32	100	45
survival ratio controls	0.32		0.45	
AV SD normalized controls	7.06	16.12	6.18	10.76
AV SD among duplicates	5.75	8.02	4.11	6.71
AV SD of survival ratio for each siRNA among the 2 experiments	12.87			

Screen

1 2

TRAIL enhancers

siRNA

TRAIL inhibitors

Log(ratio)

Figure 28

ratio	P value	annotation
8.09	0.000	H.s. plexin B1 (PLXNB1), mRNA ^{""}
9.24	0.000	H.s. SET domain-containing protein 7 (SET7), mRNA
9.89	0.001	H.s. mitogen-activated protein kinase kinase kinase 5 (MAP3K5), mRNA ^{""}
10.22	0.001	H.s. STE20-like kinase (JIK), mRNA
10.46	0.001	H.s. putative endoplasmic reticulum multispan transmembrane protein (RFT1), mRNA ^{""}
10.47	0.001	H.s. MAP kinase-interacting serine/threonine kinase 1 (MKNK1), mRNA ^{""}
10.70	0.001	H.s. mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), trans
11.29	0.002	Homo sapiens phosphatidylinositol-4-phosphate 5-kinase, type I, gamma (PIP5K1C
11.88	0.004	MAP2k5
12.31	0.005	Q62862 SER/THR FAMILY OF PROTEIN KINASES-RELATED
12.51	0.006	H.s. cyclin-dependent kinase 6 (CDK6), mRNA ^{""}
12.73	0.008	H.s. muscle, skeletal, receptor tyrosine kinase (MUSK), mRNA ^{""}
12.76	0.007	H.s. activin A receptor type II-like 1 (ACVRL1), mRNA ^{""}
12.92	0.008	H.s. Gardner-Rasheed feline sarcoma viral (v-igr) oncogene homolog (FGR), mRNA ^{""}
13.16	0.011	H.s. ribosomal protein S6 kinase, 90kD, polypeptide 5 (RPS6KA5), mRNA ^{""}
13.26	0.007	H.s. hypothetical protein FLJ21802 (FLJ21802), mRNA (mina53 related)
13.95	0.018	H.s. mitogen-activated protein kinase 7 (MAPK7), mRNA
74.00	0.008	H.s. glycogen synthase kinase 3 alpha (GSK3A), mRNA ^{""}
75.07	0.010	hypothetical protein FLJ32312 (FLJ32312),
77.75	0.007	similar to Pyruvate kinase, M2 isozyme (LOC148283),
78.62	0.004	H.s. hypothetical protein FLJ11712 (FLJ11712), mRNA
80.10	0.004	H.s. B lymphoid tyrosine kinase (BLK), mRNA ^{""}
84.64	0.003	P53
94.56	0.001	Hs. signal recognition particle 72kD (SRP72), mRNA

Figure 29

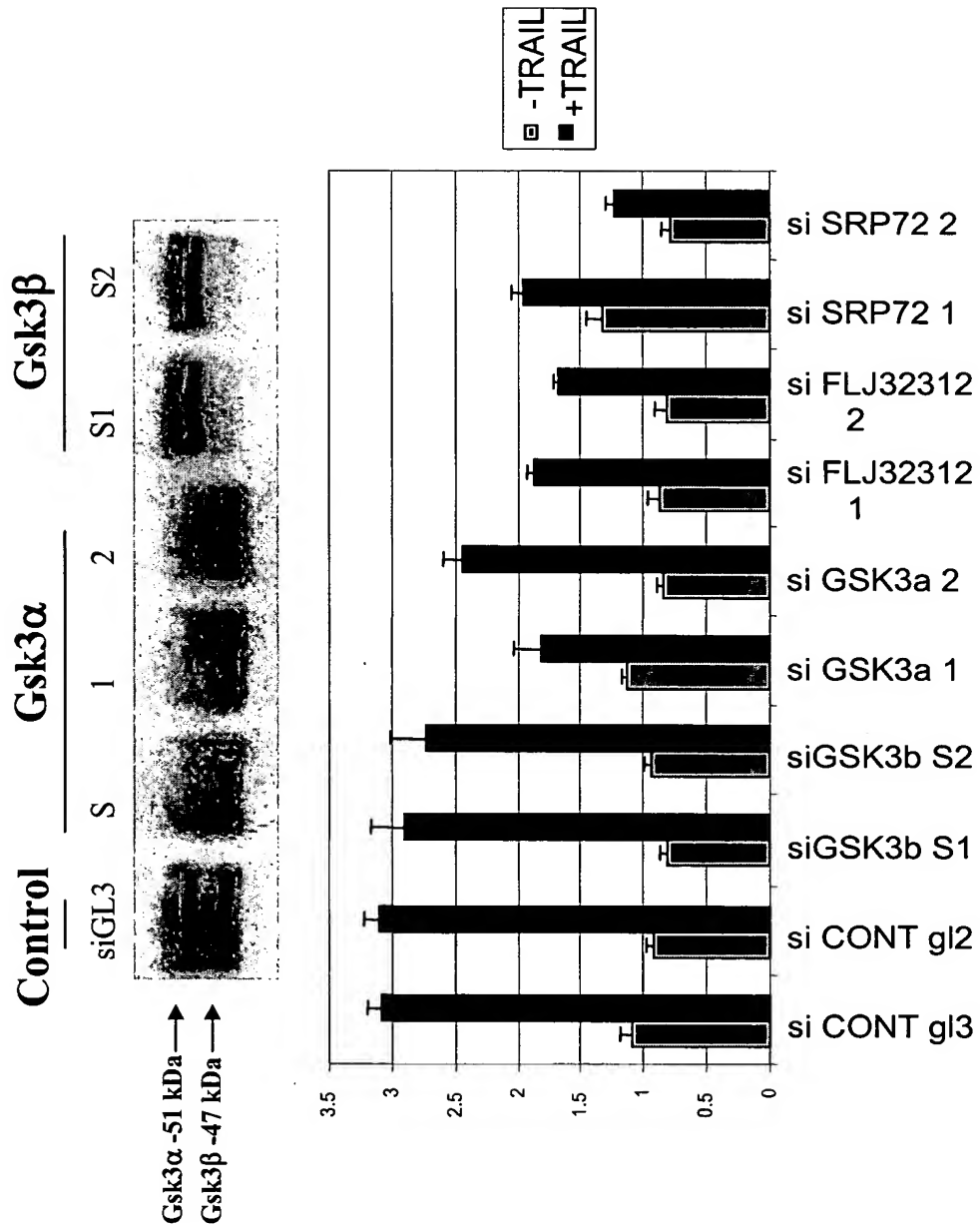


Figure 30

Global analysis

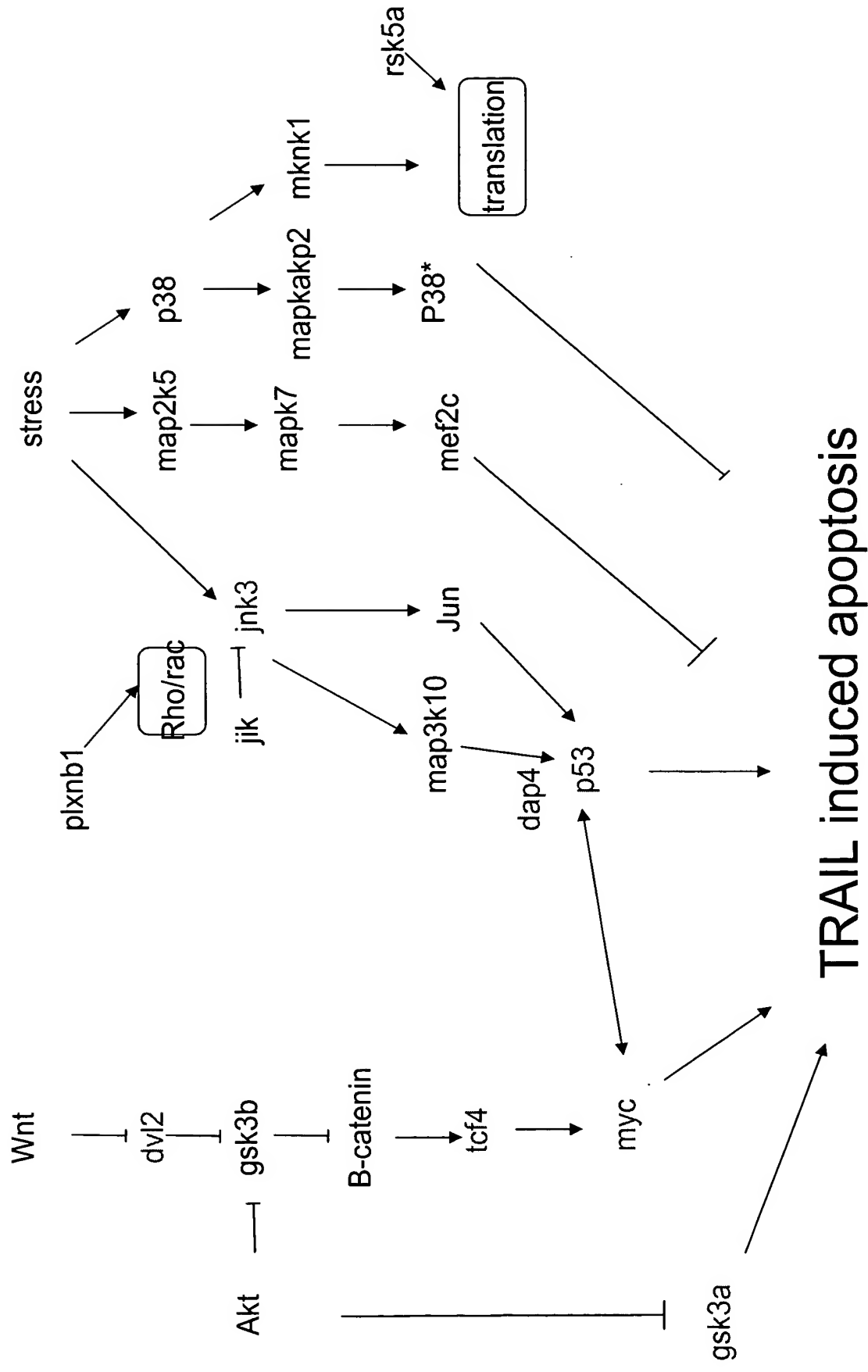
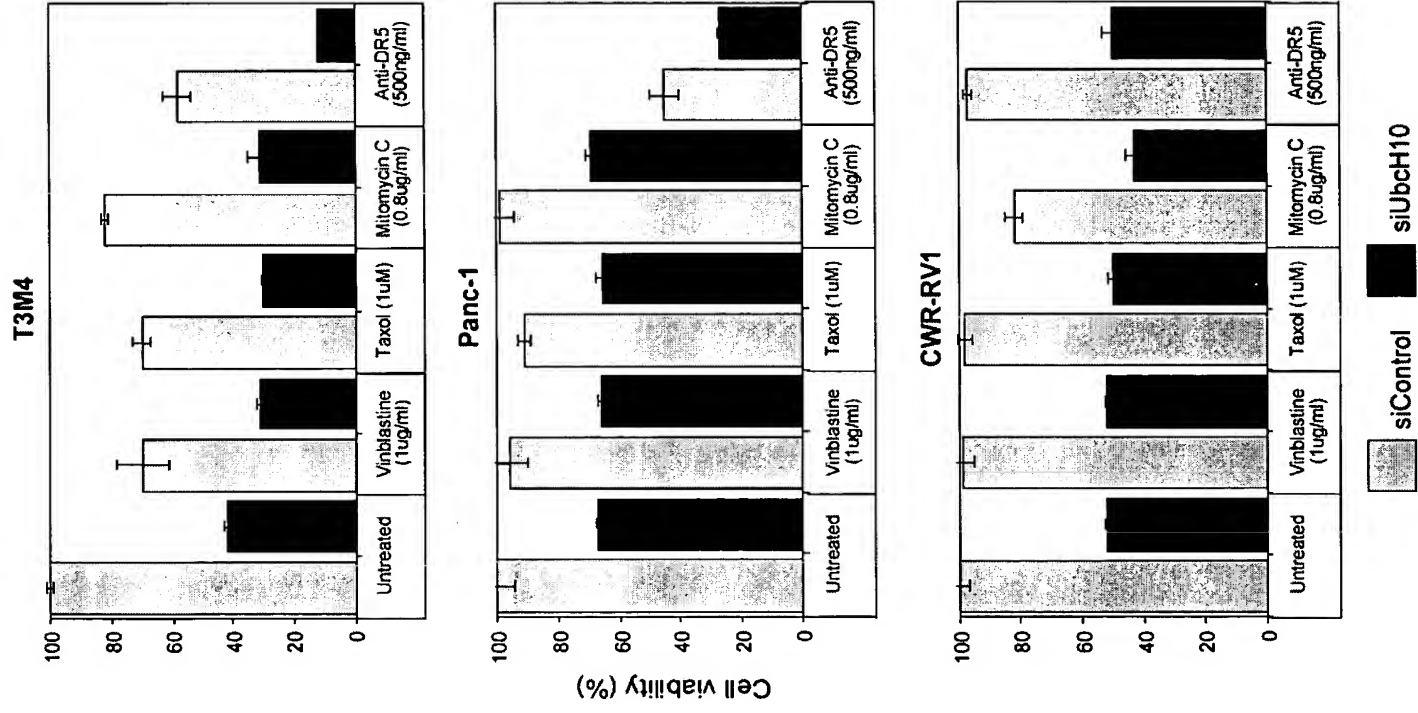


Figure 31



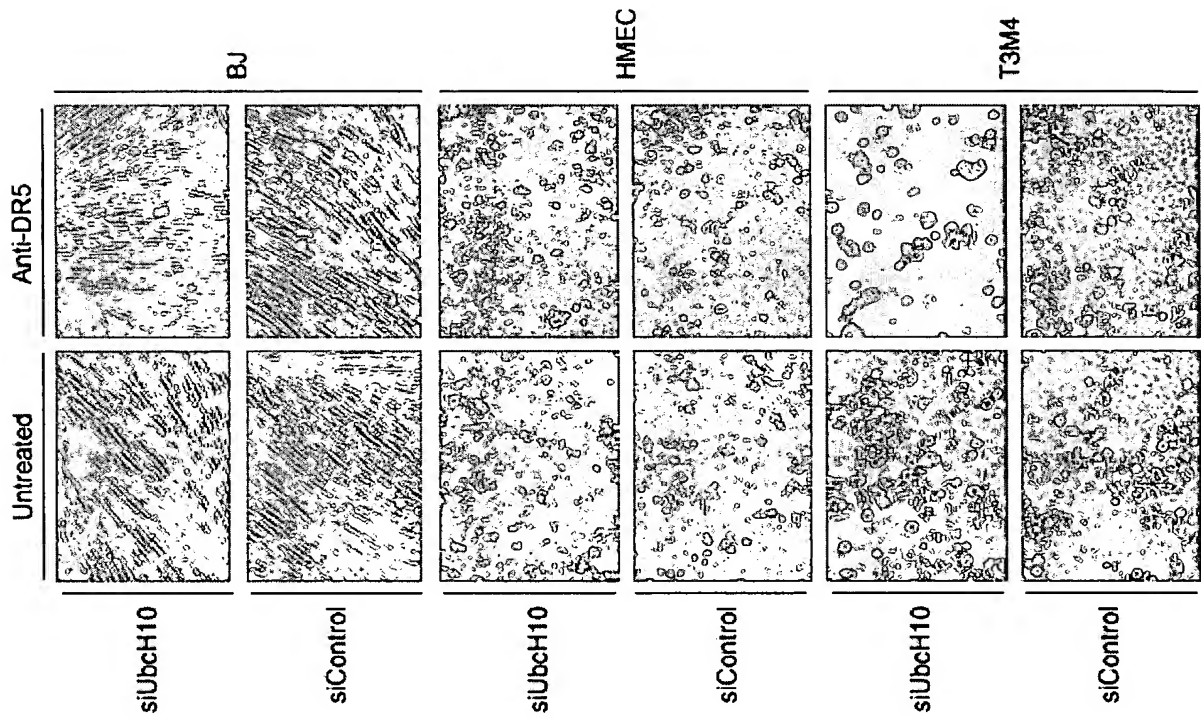


Figure 32

Sensitization of HcT116-Bax^{-/-} to TRAIL by inhibition of the proteasome

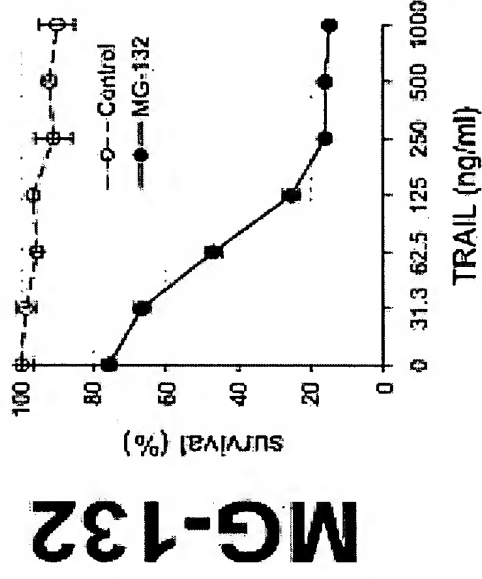
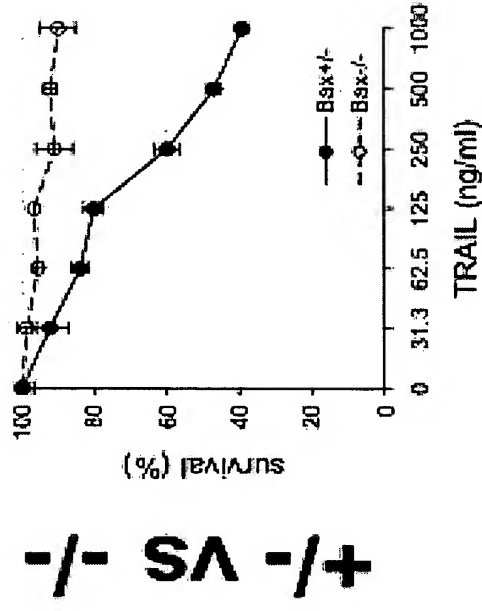
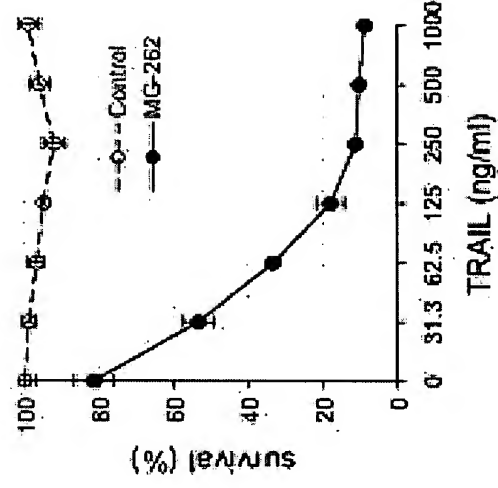
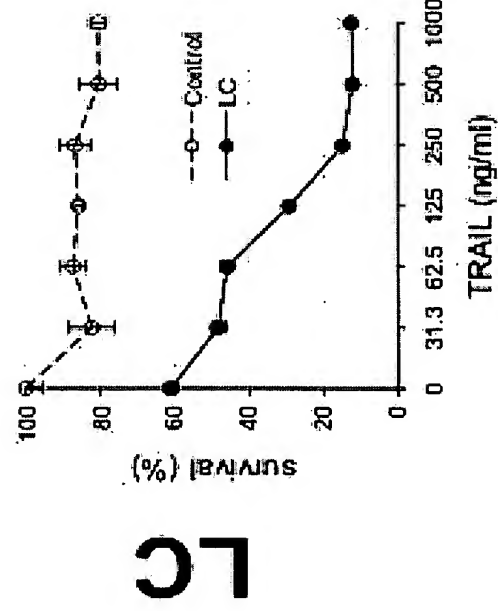


Figure 33



Measurement after 24 h, 5 μ M LC, 1 μ M MG-132, 1 nM MG-262

Figure 34
MG-262 restores the mitochondrial
apoptosis pathway

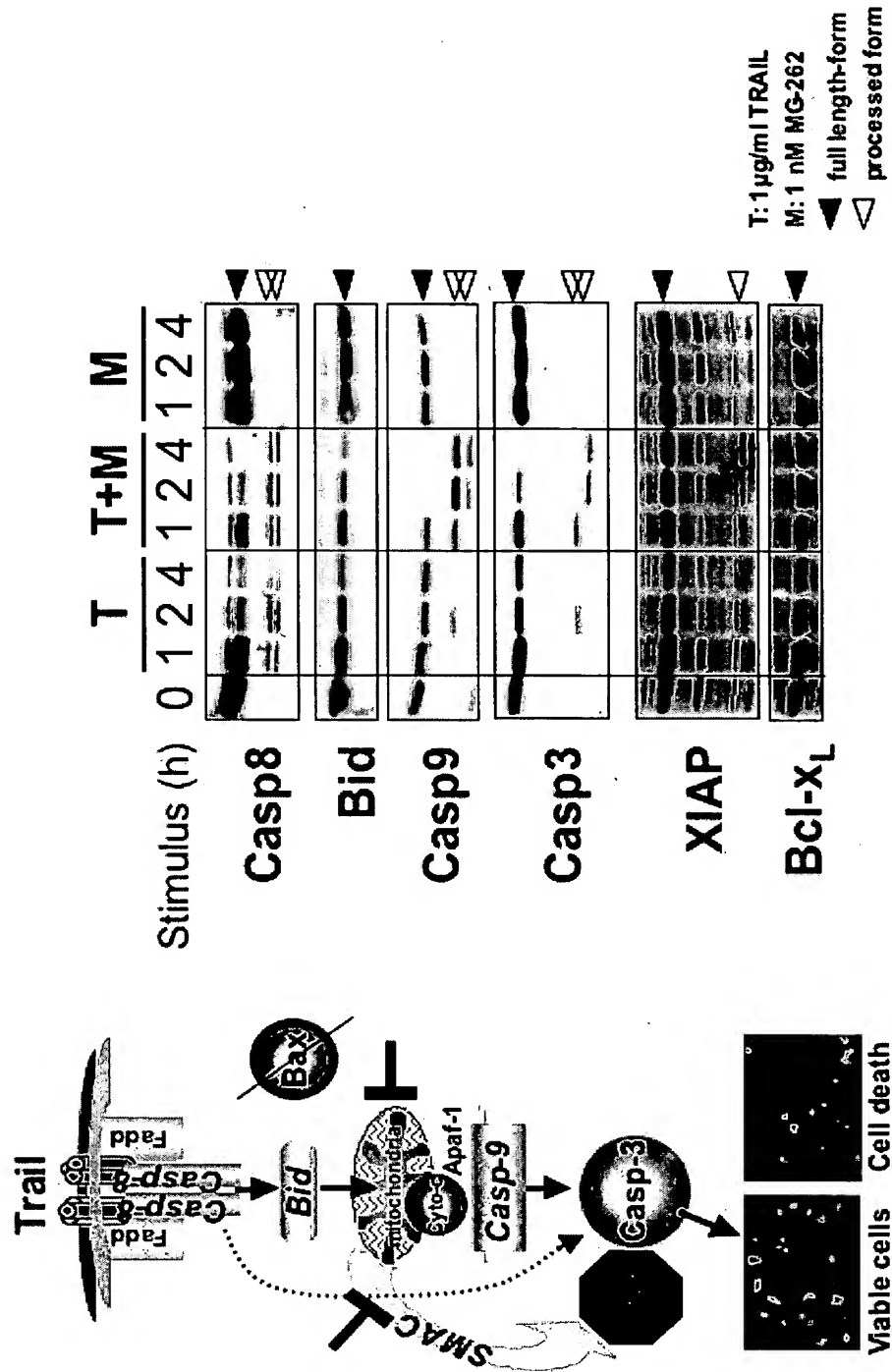


Figure 35

Sequence of DR5'A' heavy chain variable region

AAGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAACCCGGGCATCAGTGAA
GCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACACTGGGTAAA
GCAGAGGTCTGGACAGGGTCTTGAGTGGATTGGGTGTTTACCCCTGGAGGTGGTTA
TATAAAATACAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCTC
CAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGACTCTGCGGTCTATTTC
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTC
ACAGTCTCCTCA

Amino acid sequence of VH

KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIHWVKQRSGQGLEWIGWIFYPGGGYIK
YNEKFKDRATLTADKSSNTVYMELSRLTSEDSAVYFCARHEEGIFYDWGQGTTLTVSS

DNA Sequence of DR5'A' light chain variable region

GACATTGTGATGACCCAGTCTCACAAGTTCAATGTCCACATCAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAAA
ACCAGGGCAATCTCCTAAACTACTGATTACTGGGCATCCACCCGCACACTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTGT
GCAGGCTGAAGACCTGGCACTTTATTACTGTCAGCAACATTATACCACTCCATTCACGT
TCGGCTCGGGGACAAAGTTG

Amino acid sequence of VL

DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAIAWYQQKPGQSPKLLIYWASTRHTGVPDR
FTGSGSGTDYTLTISSVQAEDLALYCCQQHYTTPFTFGSGTKL